Summaries and assessments of selected studies

In the period from February to April 2015, 109 new publications have been identified, and 14 of these were discussed in depth by BERENIS. Based on the selection criteria, nine of these publications were selected as the most relevant ones. Their summaries and assessments are provided below.

1) Experimental animal and cell studies

Radiofrequency electromagnetic fields and a mouse cancer model (Lerchl et al. 2015)

In this well-described and -controlled independent replication study of a previous experiment from Tillmann *et al.* (2010), Lerchl *et al.* (2015) investigated the impact of UMTS-modulated radiofrequency electromagnetic fields (RF EMF) (0.04, 0.4, and 2 W/kg SAR) on the incidence and histopathology of tumours. The authors used an animal cancer model based on chemical mutagenesis during embryo development that primarily results in a two- to fivefold more frequent tumorigenesis in lung, liver and blood tissues. In comparison to the original study by Tillmann *et al.* (2010), more animals were used and two additional exposure levels (0.04 und 2 W/kg SAR) were included. Starting *in utero* at pregnancy day 6, lifetime exposure of female mice and their offspring with an electromagnetic field was performed. In addition, at day 14 post conception, the females were injected with the carcinogen ethylnitrosourea, at a dose of 40 mg/kg. In exposed mice, a significantly increased incidence of malignant tumour types was found in liver (starting at 0.04 W/kg) and lung (at 0.4 W/kg). These findings are mostly in agreement with the previous report of Tillmann *et al.* (2010), who also observed an impact of RF EMF on tumour growth when animals were additionally exposed to the carcinogen. Moreover, Lerchl *et al.* found a higher incidence of lymphomas at 0.4 W/kg. Strikingly, a dose-response relationship could not be established, and it can merely be speculated about the underlying mechanism leading to this tumour-promoting effect. The electromagnetic field might modulate the effect of the chemical mutagen on the metabolic level, the efficiency and accuracy of DNA repair, the immune system fighting the cancerous cells or the growth of the tumours. The results of a large study currently being performed at the National Institute of Health (NIH) in the US may help clarifying this question.

Radiofrequency electromagnetic fields and in vivo genotoxicity in mice (Zong et al. 2015)

A possible explanation for the observations made by Lerchl *et al.* (2015) and by Tillmann *et al.* (2010) was provided in the *in vivo* study by Zong *et al.* (2015). They used classic methods for the assessment of genotoxicity and oxidative stress. During one week, mice were exposed with a RF EMF (continuous 900 MHz wave, SAR ~50 mW/kg) for 4 hours/day. DNA damage was then induced by an *in vivo* application of the cancer therapeutic Bleomycin, which leads to the oxidation of molecules. The increase of the DNA fragmentation, an indicator of genetic damage, was reported to be significantly reduced in leukocytes of exposed mice compared to the control groups. In parallel, the authors analysed markers for oxidative stress (malondialdehyde concentration and superoxide-dismutase activity) in different tissues, which were also found to be changed depending on the exposure. These data suggest that radiofrequency electromagnetic field exposure might trigger systemic alterations, which in turn influence the organismic and cellular response to additional stress factors. This phenomenon is known as “adaptive response” and may play a critical role in real life where various types of stresses co-exist.

Extremely low frequency magnetic fields, radiofrequency electromagnetic fields and in vitro genotoxicity (Duan et al. 2015)

Genotoxicity of extremely low frequency magnetic fields and RF EMF were evaluated in the technically sound *in vitro* study of Duan *et al.* (2015). A spermatocyte-derived mouse cell line was intermittently exposed to different intensities of either a low frequency magnetic field (50 Hz; 1, 2 and 3 mT; 5/10 min on/off) or a RF EMF (1.8 GHz; SAR 1, 2 and 4 W/kg; GSM talk mode signal) for 24 hours. None of these exposure conditions did impact the cell viability. A significant increase of DNA fragmentation (assessed by the Comet assay) as well as of the proportion of cells exhibiting a signal for a DNA double-strand break marker (γ-H2AX) was observed in cells exposed to the highest dose of the extremely low frequency magnetic field, whereas the exposure to the RF EMF did not result in any changes. However, at the highest intensity of the radiofrequency field, a significant increase in DNA damage was observed when using a modified version of the Comet assay that also detects oxidative DNA damage. The authors concluded that both extremely low frequency magnetic fields and RF EMF may damage the genome, although through different mechanisms. Even though this conclusion supports some of the previous observations made in comparable studies, it also contradicts many others that did not find such effects. The fact that the raw data of these studies are mostly unavailable prevents an ultimate assessment of the experimental results and possible alternative explanations.

Low-frequency magnetic field exposure of immune cells (Golbach et al. 2015)

The effect of low frequency magnetic fields on immune cells has been studied by Golbach *et al.* (2015). Despite the fact that many studies on possible effects on the immune system have been published, underlying mechanisms and target structures are still unknown. The authors used an *ex vivo* model consisting of human neutrophils isolated from human blood that – upon *in vitro* activation with a chemical – produces 'NETs' (neutrophil extracellular traps). NETs are first-line defence systems to eradicate invading bacteria and other pathogens. The neutrophils were activated *in vitro* by a chemical agent, and the effect of a magnetic field (combination with four different frequencies of 320, 730, 880 and 2'600 Hz with an intensity of 300 µT) on activated as well as non-activated cells was compared. NET formation was enhanced by low frequency magnetic field exposure in activated neutrophils but not in non-activated cells. The elevated NET formation correlated well with increased antimicrobial properties, which was tested in an *in vitro* system. While the antimicrobial property is a beneficial effect, NETs also have cytotoxic effects that can lead to autoimmune diseases like arthritis. In the *ex vivo* system used in this study, apoptosis and cell death were accompanied by increased NET production. The molecular mechanism underlying this effect has been studied as well. The authors provide first evidence that low frequency electromagnetic fields enhance NET formation via an NADPH-oxidase-dependent mechanism, possibly by upregulated production of reactive oxygen species (ROS).

2) Human experimental study

Radiofrequency electromagnetic fields and brain activity during sleep: inter- and intra-individual variation (Lustenberger et al. 2015)

Pulse-modulated RF EMF, as applied in mobile phone technology, often altered brain activity during sleep in previous studies, which was measured by electroencephalography. Reproducible increases of electroencephalographic power were frequently reported for two frequency ranges characterised by typical wave patterns of the electroencephalogram (EEG) in certain phases of sleep: the sleep spindle

(13.75 - 15.25 Hz), and the delta-theta (1.25-9 Hz) frequency range. These field effects showed striking inter-individual differences. However, it is still unknown whether individual subjects react in a similar way when repeatedly exposed. Thus, Lustenberger *et al.* (2015) aimed to investigate inter-individual variation and intra-individual stability of RF EMF effects. They exposed 20 young male subjects twice two weeks apart for 30 min prior to sleep to the same RF EMF (and twice to a sham exposure) in a double-blind, randomised cross-over design. The exposure consisted of an amplitude modulated 900 MHz RF EMF (2 Hz pulse, 20 Hz Gaussian low-pass filter and a ratio of peak-to-average of 4, peak spatial absorption rate of 2 W/kg averaged over 10 g), which had also been applied by Schmid *et al.* (2014). All night high-density (128 electrodes) EEG was recorded after each exposure condition. Topographical analysis of EEG power during all-night non-rapid eye movement sleep revealed exposure related increases in delta-theta frequency range in several fronto-central electrodes, and no differences in spindle frequency range. No reproducible within-subject RF EMF effects on sleep spindle and delta-theta activity in the sleep EEG were observed. It thus remains unclear whether a biological trait of how the subjects' brains react to RF EMF exists. The authors were not able to reproduce previous findings on sleep spindle activity, and discuss different potential factors that might have contributed to this: (1) Participants performed two different learning tasks, one before and one during exposure (sham or real EMF). The word-pair declarative learning task performed prior to exposure is known to change spindle activity and might have interfered with exposure-related changes. This would mean that RF EMF effects are rather small and may easily be masked. 2) In several previous studies electrodes were applied to the subject's head prior to exposure to minimize the time between exposure and bed time. However, the used high-density EEG net acts as a Faraday's cage and therefore had to be applied after the exposure. It is still a debated issue whether possible effects might be partly due to locally induced currents in skin or cortex by electrodes during exposure. This possibility seems to be very unlikely though (Murbach *et al.*, 2014). An exposure-related increase in spindle activity in spite of attaching the electrodes after exposure was reported by Loughran *et al.* (2005).

3) Epidemiological studies

Time trends of brain tumour incidence in the Swedish National Inpatient Register (Hardell & Carlberg 2015) and the New Zealand Cancer Registry (Kim et al. 2015)

Comparing the development of disease rates over time with other factors is considered as the weakest study design in epidemiology. A good example for the limited informative value of such comparisons is the parallel decrease of birth rates and the stork population[1]. Nevertheless, time series analyses do have some informative value when considering the association between mobile phone use and brain tumours: Firstly, in many countries, the development of brain tumours over time can reliably be evaluated using available cancer registry data. Secondly, given the strong increase in the use of mobile phones over the past 20 years, a potential risk should also become manifest in the cancer rates. Thirdly, there is no other known external risk factor for brain tumours that has substantially changed during the past years and could thus have contributed to a strong increase or decrease. However, a major challenge for such time series analyses are changes in diagnostics and coding practice over time. The introduction of imaging techniques implies that more cases are diagnosed today as compared to before. All these are important aspects to be considered when interpreting the results of such studies.

[1] for example described in: Höfer T, Przyrembel H, Verleger S (2004): **New evidence for the theory of the stork**. Paediatr Perinat Epidemiol. Jan;18(1):88-92. http://www.ncbi.nlm.nih.gov/pubmed/14738551

From Swedish hospital data, Hardell & Carlberg (2015) concluded that non-specified tumours in the brain and the central nervous system (ICD-10 code: D43) have increased by 4.25% annually from 2007 to 2013. The increase was even higher in the causes of death register (23% annually). The authors assume that this is due to increasing mobile phone use. However, this analysis has a number of weaknesses that considerably limit the informative value of the study. It is not evident from the hospital data when they refer to multiple hospitalisation of the same person due to the same disease, and it is not possible to differentiate primary from secondary tumours (metastases). Furthermore, Hardell & Carlberg (2015) did not use age standardised incidence rates. Thus, a certain increase is to be expected simply because the population has continuously become older. Moreover, the presented death register data show that malignant brain tumours (code C71) have decreased to the same amount than unspecified tumours (code D43) have increased. This shows primarily that a change of the coding practice has occurred. The most likely reason for this is the marked decrease of autopsies in Sweden during the past years, resulting in tumour types more frequently being classified as unknown. As mobile phone use might predominantly affect tumours in the head region, it would have been necessary to omit tumour types in the spinal cord (code D43.4) and other/not specified locations of the central nervous system (codes D43.7 and 43.9) from the analysis. However, this has not been done. For this reason, the analysis of Hardell & Carlberg (2015) cannot provide any evidence for a correlation between mobile phone use and brain tumours.

Kim *et al.* (2015) carried out a time series analysis with New Zealand Cancer Registry data from 1995 to 2010. No increase of age standardised incidence rates for malignant brain tumours (glioma) was found at ages 10-69. Temporal and parietal lobe sites were examined separately as they are located in the area of maximum mobile phone exposure, but no increase was found in these locations either. Separate gender specific analyses for age groups with 20 year intervals found a significant decrease of brain tumours in males aged 10-29, as well as significant increases in women aged 30-49 and men at ages over 70. In summary, this study does not indicate that malignant brain tumours have increased following the introduction of mobile phones. As only about 180 tumours annually have occurred in New Zealand, this study cannot detect statistically significant small changes in the incidence rates. Small changes would be expected if only rare subtypes were affected by mobile phone exposure, or if only persons with very intensive mobile phone use had an increased risk. The study cannot draw any conclusions for latency periods of more than 15 years either, because less than 10% of the population had a mobile phone subscription before 1995. From 2007 onwards, the number of mobile phone subscriptions exceeded the number of inhabitants in New Zealand.

Mobile phone use during pregnancy and birth outcomes (Baste et al. 2015)

Based on data of the Norwegian Mother and Child Cohort Study (1999–2009), Baste *et al.* (2015) investigated maternal mobile phone use during pregnancy and paternal mobile phone use prior to conception. The cohort was linked to the Norwegian Medical Birth Register to obtain information about all singleton pregnancy. Reproductive health outcomes studied were congenital malformations, perinatal mortality, low birth weight, preterm birth, born small for gestational age and preeclampsia during pregnancy. The authors did not find an association between maternal mobile phone use and adverse pregnancy outcomes. For preeclampsia, the authors even found a significantly reduced risk. Regarding paternal mobile phone use, two associations were identified, yet neither consistent nor pronounced: fathers' testis exposure was associated with a borderline increased risk of perinatal mortality, and mobile phone exposure of head or testes was related to a slightly decreased risk of preeclampsia during pregnancy. A positive feature of this study is the prospective design – the mothers were interviewed in gestational weeks 15 and 30, thus at a time when they could not possibly know anything about potential reproductive outcomes. In addition, the

large sample size with more than 100'000 births included in the analysis (and almost 75'000 with paternal information) allows drawing reliable conclusions. However, it remains unclear how relevant maternal mobile phone use was for foetal exposure since mobile phone radiation to the foetus is minimal during maternal mobile phone calls. Furthermore, the study cannot make any statements about the impact of RF EMF on spontaneous abortions occurring before gestational week 15.

4) Dosimetric study

Specific absorption rate in in vitro experiments and in realistic scenarios (Schmid & Kuster 2015)

The study of Schmid & Kuster (2015) is dealing with the discrepancy between the maximum values of the specific absorption rate (SAR) used in *in vitro* experiments in most cases, and the maximum local SAR values occurring when using mobile phones in realistic scenarios. For this, 80 studies on *in vitro* experiments published after 2002 were assessed with regard to the maximum SAR values used. In 51 studies, applied exposure levels were 2 W/kg or less. 2 W/kg per 10 g tissue corresponds to the maximum SAR allowed for the population, averaged over 10 g body tissue. In order to determine in higher resolution the maximum local SAR values occurring in realistic scenarios, various layered planar numerical models and a numerical model of a commercially available mobile phone were evaluated with an anatomical head model by applying the finite-difference method. The spatial resolution in the different models was smaller than 0.25mm. The transmitting power was adjusted in such a way that the SAR value averaged over 10 g of tissue was 2 W/kg. As a result of the different absorption characteristics of the modelled tissues and their different distances to the antenna, the local absorption of the radiation is very variable, and can substantially exceed the spatial mean value of 2 W/kg over 10 g of tissue for single cells. In the case of the anatomical head model, the calculated exposure of skin, blood and muscle tissues was as high as 40 W/kg at the cell level. It is stated that cell studies reporting minimal or no effects with maximal relatively uniform SAR values of 2 W/kg (with deviations of ± 30%) or less may be of only limited value for risk assessment. For future studies, the authors recommend the use of higher maximum SAR values, and rigorous control of the corresponding temperature increase caused by the microwave within the cell medium. In principle, the deviations detected in this study and the corresponding recommendations for future studies are correct. This means that in reality when using a mobile phone, the specific absorption rate of certain cells can exceed 2 W/kg, and studies with higher SAR values are of relevance for risk assessment. When using higher SAR values it is indispensable to control the temperature accordingly. Furthermore, it should be globally re-considered which exposure measures are most relevant for biological processes. A cell culture consisting of one cell type within a culture medium represents at best an approximation of the environment and the conditions found by a cell in the tissue.

5) Review

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) of the European Commission has published an opinion on potential health effects of exposure to electromagnetic fields in January 2015:

SCENIHR (2015): Opinion on potential health effects of exposure to electromagnetic fields (EMF). Scientific Committee on Emerging and Newly Identified Health Risks. European Commission. http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_19_en.htm

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Additional information related to the Swiss expert group on electromagnetic fields and non-ionising radiation (BERENIS) and a list of abbreviations can be found at
http://www.bafu.admin.ch/elektrosmog/01095/15189/index.html?lang=en

Link to list of abbreviations (pdf)