Summaries and assessments of selected studies

In the period from end of April to beginning of August 2019, 103 new publications have been identified, and nine 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

Millimeter waves and DNA damage (Koyama et al. *2019)*

Koyama *et al.* (2019) investigated in this small but technically sound study the induction of DNA damage by RF-EMF in the frequency range of millimeter waves that might be used for 5G in future. The authors exposed human eye cells (corneal epithelial and lens epithelial cells) to unmodulated 40 GHz millimeter wave EMF at a power density of 10 W/m2 (corresponding to the whole body limit) for 24 hours. Performing Comet and micronucleus assays, DNA damage levels in the cell nucleus and faulty repair events and/or problems in the DNA replication were assessed, respectively. No significant changes were observed in both analytical approaches for the two cell types tested.

Thus, this study provides no evidence for direct or indirect DNA damage caused by 40 GHz millimeter wave exposure. A strength of this study is the fact that several controls were used (control for potential incubator effects, sham exposure, positive control). In addition, a well-characterized exposure system was used to control for thermal effects, supported by the absence of increased levels of heat-inducible stress proteins ("heat shock protein", Hsp).

Global search for biomarkers for millimeter wave exposure (*Le Pogam* et al. *2019)*

The cell culture study by Le Pogam *et al.* (2019) is presented here primarily because of its novel hypothesis-free experimental approach, which has not been used with regard to RF-EMF in this way before. Human skin cells (keratinocytes) were exposed to a 60.4 GHz RF-EMF for 24 hours with high power density (200 W/m^2 = 20 times higher than whole body limit). Subsequently, the complete set of small metabolic molecules (metabolome) and lipids (lipidome) was analyzed by mass spectrometry. A distinction was made between molecules outside (extracellular, culture medium and discharged) and inside the cell (intracellular). With this approach, it was possible to reproducibly detect more than 15,000 molecules, providing a good overview of the entire metabolic activity. Approximately 2,600 of these were altered in more than two ways in bioinformatic analysis and can be considered potential biomarkers for millimeter wave effects. However, only a small fraction of the molecules can be clearly identified by determining the mass, and the relative changes were not very pronounced in general. With regard to quantity, exposure-related changes were most pronounced for the intracellular lipidome and the extracellular metabolome. In the lipidome, the authors found components which have a structural and also a signaling function in the cell membrane. This observation was also made in the extremely low frequency range recently (see BERENIS Newsletter No. 18). A remarkable amount of potential metabolic biomarkers were found in the culture medium. The authors interpret this as an indication of increased permeability of the cell membrane due to exposure to millimeter waves. From a biological point of view, this explanation seems reasonable, and changes in channel activity due to exposure have already been observed in previous studies. However, there is no control that shows that these results are not due to direct thermal effects, since significant heating occurs at such high

power density. Furthermore, the experimental conditions and the exposure dosimetry are not sufficiently controlled. Further analyses and independent replication will be necessary to exclude these uncertainties and to assess the relevance of these findings.

The cell culture study by Le Pogam *et al.* (2019) is presented here primarily because of its novel hypothesis-free experimental approach that has not been used with regard to RF-EMF before. Human skin cells (keratinocytes) were exposed to a 60.4 GHz RF-EMF for 24 hours with high power density (200 W/m^2 = 20 times higher than whole body limit). Subsequently, the complete set of small metabolic molecules (metabolome) and lipids (lipidome) was analyzed by mass spectrometry. A distinction was made between molecules outside (extracellular, culture medium and secreted compounds) and inside the cell (intracellular). The authors could reproducibly detect more than 15,000 molecules, providing a good overview of the entire metabolic activity. Performing bioinformatic analyses, approximately 2,600 of them were found to be altered and can be considered potential biomarkers for millimeter wave effects. However, only a small fraction of these molecules could be clearly identified by mass spectrometry and the relative changes were generally not very pronounced. With regard to numbers, exposure-related changes were most pronounced for the intracellular lipidome and the extracellular metabolome. For instance, the authors found components in the lipidome, which have a structural as well as a signaling function in the cell membrane. Notably, similar observations were recently made upon exposure to ELF-MF (see BERENIS Newsletter No. 18). A remarkable amount of potential metabolic biomarkers was found in the culture medium. The authors interpreted this as an indication of increased permeability of the cell membrane due to exposure to millimeter waves. From a biological point of view, this explanation seems reasonable, and changes in channel activity due to exposure have already been observed in previous studies. However, the control is missing to exclude direct thermal effects, since significant heating might occur at such high power density. Hence, the experimental conditions and the exposure dosimetry are not sufficiently controlled. Further analyses and independent replication will be necessary to exclude these uncertainties and to assess the relevance of these findings.

Extremely low frequency magnetic fields and neuronal differentiation (*Özgün* et al. *2019)*

The study of Özgün *et al.* (2019) investigated effects of ELF-MF on neuronal differentiation, which has various parallels regarding cellular signaling pathways involved in neurodegeneration. The hypothesis of this study was that neuronal differentiation is influenced by proteins that are sensitive to magnetic fields, namely ion channels or transferrin. Neural progenitor cells were differentiated for five days while exposed to ELF-MF (1 mT, 50 Hz). The differentiation was more efficient with magnetic field exposure, resulting in enhanced levels of neuronal markers and neurite outgrowth. The increased expression of the *c-fos* gene indicates increased calcium concentrations in the cell and supports the finding of increased differentiation; however, calcium concentrations were not measured in the cell. According to the authors' hypothesis, an increased calcium influx into the cell via NMDA receptor (N-methyl-D-aspartate) may be involved in the effect of the magnetic field. Neutralization of this effect by chemical blocking of the NMDA receptor indirectly showed that this receptor is involved in the differentiation and effects of the magnetic field.

The findings are of interest with regard to the development and therapy of neurodegenerative diseases such as Alzheimer or Parkinson. In addition, the study shows that ELF-MF (1 mT, 50 Hz) above the ICNIRP regulatory limit activate ion channels that have been shown to be involved in differentiation. Interestingly, the effects occur during neuronal differentiation, but are unlikely to occur in mature neurons.

Radiofrequency electromagnetic fields and neuronal differentiation (von Niederhäusern et al*. 2019)*

Similar to the study of Özgün *et al.* (2019, see above), effects of EMF on neuronal differentiation were also investigated by Niederhäusern *et al.* (2019), in this case with neuroblastoma cells. This study focused on the impact of RF-EMF exposure (935 MHz, 4 W/kg, 217-Hz pulse modulated, 2 min on/2 min off, for 24 hours). In addition to signaling pathways involved in differentiation and neurodegeneration, the authors investigated oxidative stress and its resulting potential effect on cellular respiration. Impairments of mitochondrial functions have been described for neurodegenerative diseases such as Alzheimer or Parkinson. RF-EMF had an effect on differentiation in neuronal cells, and a trend (non-significant) towards increased activation of the proteins involved in differentiation, namely ERK1/2 and Akt was observed. Proteins of the Wnt signaling pathway were not regulated by RF-EMF. RF-EMF exposure had no effect on mitochondrial respiration under physiological conditions. On the other hand, glucose deprivation leading to cellular stress impaired the maximum cell respiration in RF-EMF-exposed cells. This effect was supported by the fact that RF-EMF caused increased oxidative stress in the cell (measured via GSH concentration). Mitochondrial markers for fusion and fission of mitochondria, which are responsible for the activity of these cell organelles, were not affected by RF-EMF exposure. Under physiological conditions, only trends for an impairment of neuronal differentiation were observed. However, stress, such as lack of glucose, led to oxidative stress and impaired cell respiration in RF-EMF exposed cells.

2) Epidemiological studies

First results of the international Cohort Study of Mobile Phone Use and Health (COSMOS) in Sweden and Finland (Auvinen et al. *2019)*

Many of the existing epidemiological studies on health risks related to RF-EMF from mobile phone use rely on retrospectively collected usage data, i.e. study participants being asked about their usage habits regarding duration and frequency of mobile phone calls, often dating back several years. This can lead to systematic errors due to differential recall of healthy and diseased study participants. For instance, ill participants might overestimate their exposure as they connect it to their disease. Such bias cannot occur in the prospective international COSMOS cohort study, which started in 2007 and has now published first results based on more than 24,000 participants from Sweden and Finland (Auvinen *et al.* 2019). Data on mobile phone use were collected at the very beginning of the study using a written questionnaire. In addition, also at the start of the study, objective data on call duration using the GSM (2G) and UMTS (3G) network were collected from the mobile phone providers for a period of three months. After four years, the study participants answered follow-up questionnaires investigating whether they suffered from symptoms such as headaches, tinnitus or hearing loss. With regard to tinnitus and hearing loss, the study did not find any associations with mobile phone use. The group of participants with the longest call times (>276 min/week) reported new onset of headache 13% more frequently than the group with the lowest amount of weekly call time. The exposure-response relationship was close to being statistically significant (p=0.06). The association tended to be more pronounced for UMTS use (OR=1.16, 95% confidence interval: 0.93-1.46) than for GSM use (1.06, 95% CI: 0.89-1.26).

In addition to the prospective study approach, major strengths of this study are the very large number of study participants and the use of objective data from mobile phone providers. According to the authors, the fact that the association with headaches was mainly found for the participants making calls in the UMTS network and not for those in the GSM network indicates that observed associations

were not caused by RF-EMF. When making calls in the older GSM network, exposure is about 100 to 500 times higher than in the UMTS network[1]. Correspondingly, higher risks would have been expected for GSM users than for UMTS users. Thus, it seems more likely that other (unknown) factors associated to intensive mobile phone use play a role regarding new onset of headache.

Extremely low frequency magnetic fields from high-voltage overhead power lines and neurodegenerative diseases in Northern Italy (Gervasi et al. *2019)*

The aim of the population-based case-control study by Gervasi *et al*. (2019) was to investigate a potential association between exposure to ELF-MF from high-voltage overhead power lines and the occurrence of neurodegenerative diseases such as Alzheimer and Parkinson in the Milan metropolitan area (Italy). The distance of the residential address to the nearest high-voltage overhead power line (>30kV) was used as a proxy for exposure to ELF-MF. The study included cases diagnosed between 2011 and 2016 (Alzheimer: n = 9,835, Parkinson: n = 6,810), and four control subjects per patient. The authors found a slightly increased disease risk for persons living in a distance of less than 50 m from a high-voltage power line compared to persons whose residential address was at least 600 m away from a high-voltage power line. However, the identified associations were not statistically significant.

The strengths of the study are the large size of the population studied, and the fact that it accounted for important confounding factors such as proximity to high-traffic roads. Interesting is the result of a validation analysis done with regard to diabetes mellitus (positive control outcome). As expected, this disease was associated with proximity to high-traffic roads, but not with proximity to high-voltage power lines. This is the third study conducted on this topic worldwide. Similar to the two previous studies from Switzerland (Huss *et al.* 2009) and Denmark (Frei *et al.* 2013), the present study found a weak indication that Alzheimer's disease may occur more frequently in the immediate vicinity of high-voltage power lines.

3) Review article

Public exposure to radiofrequency electromagnetic fields in Europe: an updated systematic review (Jalilian et al. *2019)*

In May 2019, Jalilian *et al.* (2019) published a systematic review of public everyday exposure to RF-EMF in Europe, based on available literature published until July 2018. Mean RF-EMF exposure in homes, schools and offices was between 0.04 and 0.76 V/m, whereas mean outdoor exposure values ranged between 0.07 and 1.27 V/m. The main contribution generally originated from mobile phone base stations. Exposure increased with increasing urbanity. In homes, the average exposure was less than 0.3 V/m. Exposure was highest in public transport (0.5 V/m and above). This review found no evidence for an increase of exposure between 2012 and 2016.

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

List of abbreviations (pdf)