Summaries and assessments of selected studies

In the period from February 2016 to April 2016, 100 new publications have been identified, and 11 of these were discussed in depth by BERENIS. Based on the selection criteria, four of these publications were selected as the most relevant ones. Their summaries and assessments are provided below. In addition, this newsletter contains an evaluation of the first partial results of a study published in May and June 2016 (Wyde *et al.* 2016).

1) Experimental animal and cell studies

Mobile phone radiation and cancer risk in rats – first partial results of a large animal study conducted in the US (Wyde et al. 2016)

In May and June 2016, first results of the largest study investigating whether mobile phone radiation increases cancer risk in rats and mice were published. This study - a bioassay, and thus lifetime exposure of animals – was conducted by the US National Toxicology Program (NTP) on behalf of the US Food and Drug Administration (FDA). Due to strict guidelines in experimental procedure, dosimetry and analyses, the quality of the study is excellent. The results published (rat experiments only) so far refer exclusively to investigations of gliomas in the brain and schwannomas of the heart of rats. The latter is relevant because tumours of the acoustic nerve are also schwannomas (vestibular schwannomas), tumours that have been linked to mobile phone use in epidemiological studies. The complete results of the study are expected to be published end of 2017. The rats were exposed to GSM and CDMA signals (900 MHz, SAR values 0, 1.5, 3 and 6 W/kg) in 10 min intervals (10 min on, 10 min off) for 18 hours per day and seven days per week. The cumulative exposure during 24 hours was 9 hours. The animals were able to roam freely, therefore the whole body has been exposed. This is in contrast to many other studies using a so-called 'Ferris wheel' exposure, where the animals were exposed or sham-exposed for a few hours per day in narrow tubes, and usually only specific parts of the body were irradiated, e.g. the brain. Starting already before birth (exposure of the dams) on day five after conception, the animals were exposed or sham-exposed life-long. From day 21 to day 34, the pups were kept in groups of three animals (maximum), and they were separated by sex. Afterwards, the rats were kept individually. The field strengths were adapted to the growth of the animals, as they absorb the irradiation differently depending on their size. For each exposure condition (0, 1.5, 3 and 6 W/kg), 90 male and 90 female animals were analysed.

Compared to the male control group, more malignant gliomas were found in the brain of the exposed male rats, though the number of cases was small (0-3 cases per exposure group). This effect of exposure was not observed in the females. Increased case numbers of heart schwannomas in exposed male rats were also found, although being only statistically significant for the highest exposure group. However, there was a trend for the occurrence of more schwannomas with increasing exposure dose. Analysis of female rats did not reveal any difference regarding the number of animals with schwannomas in the exposed group and the sham-exposed control group. The authors concluded that the increased occurrence of gliomas and schwannomas of the heart in male rats is probably due to the exposure, with the association between exposure and occurrence of schwannomas being more robust compared to the gliomas.

In the view of BERENIS, the study raises various points for discussion. The number of diseased animals was small, and EMF effects mainly occurred in male rats. One might argue that the results are random findings. However, differences between gender, and especially an increase in tumours in

male animals, are frequently found in toxicological animal studies[1] and do not question the results *per se*. A comparison with historical controls is important to assess the results even though a control with sham-exposed animals is essential. Historical controls of NTP toxicological investigations showed a (glioma) tumour rate of 0-8%. Furthermore, they showed that the probability for the formation of malignant tumours is ten times higher in male rats compared to females. The gender differences in the occurrence of malignant schwannomas in the heart were observable as well, even though less pronounced compared to the development of gliomas. The spontaneous incidence of cancer in female rats might be very small, so that the statistical power is not sufficient despite the large number of animals used in this study.

The longer survival time of the exposed animals was an unexpected finding of the study. This raises the question whether the animals in the control group had a smaller chance to develop tumours because of their shorter life span. However, in this case, one would expect to see an increase in hyperplasias (preliminary stage of a tumour) in the control group, which was not observed in this study. From a biological point of view it is striking that glial cells were affected by exposure (Schwann cells are glial cells of the peripheral nervous system), as epidemiological studies related to mobile phone use previously indicated an increased risk of tumour formation of the same brain cell type. Notably, the reports published now have not yet been subject to a peer-review process of a renowned scientific journal, but have been scientifically evaluated by invited experts. Their comments have been published in the appendix of the reports. Furthermore, the study was presented at the BioEM conference in Ghent/Belgium in June 2016. The presentation is publicly accessible[2] and contains additional information regarding thermal effects, survival times and DNA damage.

In summary, the BERENIS group concludes: With regard to mobile phone exposure and cancer, these animal studies are the most comprehensive ones that have been conducted to date. The scientific quality and the standard of the laboratory techniques are exceptionally high. Therefore, the experiments are of great scientific relevance, and important in terms of health policy. The finding that mobile phone exposure increases tumour risk in animal experiments has not been observed in most of the previous lifetime animal studies. The yet unpublished results of the mouse experiments and the findings regarding potential DNA damage remain to be evaluated in order to make more precise assessments of the relevance for human health. One needs to consider that the EMF exposure used in the experiments is not directly comparable with real-life exposures of humans. The whole body exposure of up to 6 W/kg used here leads to an increase of the core temperature of the animals of up to 1°C. In Switzerland, the regulatory limit for whole body exposure is 0.08 W/kg, which does not cause a noticeable temperature increase. Mobile phone use can cause SAR values up to 2 W/kg locally at the ear or hand.

Radiofrequency electromagnetic fields and autophagy in the brain of mice (Kim et al. 2016)

This *in vivo* study investigated effects of RF-EMF (835 MHz, 4 W/kg) on the brain of mice (C57BL/6; age at the onset of the study: 6 weeks). The animals were exposed or sham exposed for 5 hours per day for 4 and 12 weeks. Thereafter, microarray analyses were performed. Genes that were expressed higher or lower were subsequently evaluated by quantitative PCR (polymerase chain reaction). In the hippocampus and striatum regions of the brain, an increased expression of genes relevant for

[1] Kadekar et al. (2012): Gender differences in chemical carcinogenesis in National Toxicology Program 2-year bioassays. Toxicol Pathol. 2012 Dec;40(8):1160-8. http://tpx.sagepub.com/content/40/8/1160.long
[2] https://ntp.niehs.nih.gov/ntp/research/areas/cellphone/slides_bioem_wyde.pdf

autophagy[3] were found in RF-EMF-exposed animals compared to sham-exposed control animals after 12 weeks of exposure. These results are based on analyses of several autophagy genes. Furthermore, specific vacuoles that were generated during autophagy were found in cells of these brain regions whose size and number were significantly increased in the EMF-exposed animals. In addition, a significant increase in so-called 'autophagosomes' and 'autolysosomes' was observed. These effects indicate an increase in autophagy after subchronic EMF exposure, which can be interpreted as adaptation to stress and subsequent protective measures.

Extremely low frequency electromagnetic fields and communication between neurons in the brain (Sun et al. 2016)

Biological effects of ELF-MF might be caused by interactions with elements in the cell membrane. Neuronal cells (neurons) are well suited for investigations of potential mechanisms regarding the impact of ELF-MF on communication between neurons in the brain, a process that is influenced by exo- and endocytosis. Endocytosis is a cellular process in which cells absorb particles or liquids from outside by engulfing it with the cell membrane. This process also regulates the composition of the cell membrane and the distribution of receptors. These investigations were performed with organotypic brain slices from exposed animals (50 Hz, 1 mT, or control animals) by using electrophysiological methods. For this reason, calcium currents and membrane capacitance were measured at the central nervous synapse (calyx of Held). The process of endocytosis was found to be increased in the animals exposed to the magnetic field. In contrast, exocytosis (expelling of particles or liquids) was not altered. ELF-MF exposure of female and male C57 mice for 8-10 days significantly increased the calcium influx upon electrical stimulation. ELF-MF exposure also caused a potentiation of short-term neuronal plasticity, which regulates the release of neurotransmitters. According to the authors, these findings may be caused by increased calcium channel expression (subtypes P/Q, N and R) at the presynaptic nerve terminal and an increased calcium influx.

Extremely low frequency electromagnetic fields and impact on the regulation of cell proliferation (Martínez et al. 2016)

Based on previous observations showing a stimulation of the proliferation of specific cancer cells by exposure with extremely low frequency magnetic fields, Martínez *et al.* (2016) investigated the interplay of ELF-MF and basic cellular mechanisms for cell proliferation *in vitro*. For this purpose, a human brain cancer cell line was used, in which a 10% increase in the cell number was found after 3 days of exposure to an ELF-MF (50 Hz, 100 µT, 3 hours on/3 hours off). This correlated well with the finding of an increased number of cells in the DNA replication and cell proliferation phase in the exposed cell culture after one or two days. Furthermore, the authors showed that cell cycle-regulating proteins were modified after two hours of exposure, and that a transient activation of two central signal transduction pathways for cell proliferation (p38, MAPK) was observed already after 15 minutes. Although the activation of both signal pathways is required for the stimulation of cell proliferation by the ELF-MF, interestingly, only one of them reacted to the inhibition of free radical production. This indicates that the exposure-related release of free radicals by the cell has a signalling effect, which has a direct impact on cell proliferation. In summary, the authors suggest that the exposure of cells to ELF-MF might cause an – at least transient – activation of various signalling pathways. This would imply that a cell type specific response to the exposure could be expected,

[3] Autophagy means "self-eating" and protects the cell from functional disorders by disintegrating defect or even damaging cell components or pathogens. They are isolated in specialised cell organelles, degraded and finally returned into the cell metabolism. Overly stressed or deficient autophagy can lead to diseases.

which could have different consequences depending on the presence of other environmental factors or genetic preconditions. This complicates a prediction of potential health relevant impacts on cells in the human body based on these observations. Nevertheless, the results provide an interesting mechanistic starting point for further studies.

Extremely low frequency electromagnetic fields and development of neural stem cells (Ma et al. 2016)

Ma *et al.* (2016) investigated effects of ELF-MF (50 Hz, 1 mT) exposure in neural stem cells of mice and on their neurogenesis *in vitro*. The authors found that four hours of exposure per day during one, two and three days promotes the proliferation and the preservation of stem cell characteristics of the neural stem cells. However, the exposure during the induced differentiation process to neuronal cells led to increased development of neurons. The development of astrocytes, which have structural and nutritional functions in the brain, was not affected. Exposed neurons did not develop more neurites, but longer ones with more branching points. The increase of a specific calcium channel in the cell membrane, whose absence eliminated all effects of the ELF-MF on the differentiation process, was an interesting finding from a mechanistic point of view. This leads to the conclusion that an ELF-MF influences cellular calcium concentration, which in turn affects neuronal development. This could influence the regeneration capacity of the brain. According to the authors, it is not clear whether this has positive or negative implications, as even small changes during embryonic development can have serious consequences in adults. Notably, previous investigations (Chen et al. 2014[4], see BERENIS-Newsletter Nr. 1) have found a contrary influence of RF-EMF on neuronal development. Whereas the causal relations are still unknown, the results of this and similar studies could provide an explanation for the observation of cognitive and pathologic effects of RF-EMF as well as of ELF-MF.

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http://biorxiv.org/content/early/2016/05/26/055699 (original version, posted May 26, 2016)
http://biorxiv.org/content/early/2016/06/23/055699 (updated version, posted June 23, 2016)

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/13893/15174/16478/index.html?lang=en

Link to list of abbreviations (pdf)