Summaries and assessments of selected studies

In the period from November 2015 to January 2016, 129 new publications have been identified, and 11 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. In addition, this newsletter contains an evaluation of a study published in March 2016 (Andrianome *et al.* 2016).

1) Experimental animal and cell studies

Radiofrequency electromagnetic fields, oxidative stress, inflammatory response and DNA damage in rats (Megha et al. 2015)

This in *vivo study* focussed on investigations on the development of oxidative stress, inflammatory response and DNA damage following exposure of male Fischer 344 rats (inbred strain) at frequencies of 900, 1800 and 2450 MHz. Whole body SAR values were around 0.6 mW/kg and very similar at all frequencies (0.59, 0.58, 0.66 mW/kg). The duration of exposure was 60 days (two hours per day, five days per week). The rats were randomly assigned to four exposure groups including sham exposure. It is not mentioned whether the experiment had been blinded, and no details regarding the dosimetry are provided. The values for the whole body SAR represent a worst case scenario. SAR values in specific body parts or organs such as e.g. the brain are not specified. The measured markers for oxidative stress (MDA = malondialdehyde; PCO = protein carbonyl; CAT = catalase) and inflammation (TNFα, IL-2, IL-6, IFNγ) were significantly increased after microwave exposure in the brain (hippocampus) with the effect being frequency-dependent. The antioxidants (SOD = superoxide dismutase und GSH = reduced glutathione) decreased with increasing frequency indicating that oxidative stress and inflammatory processes were related to the exposure. Again, the observed effects increased with increasing frequencies. DNA double strand breaks were assessed using the comet assay. The migration of DNA fragments in the comet tail was significantly increased after EMF exposure. The authors suggest that the observed DNA damages in cells of the hippocampus were induced by oxidative stress. It is remarkable that effects occurred at such low radiation levels. Furthermore, it is a surprising and rather novel finding that effects depend on the frequency of the radiation at about equal SAR values. These findings are relevant for human health. However, more experiments are needed to evaluate these effects further. This would require a replication of the experiment with improvements regarding blinding of experiments and providing a more detailed dosimetry.

Extremely low frequency magnetic fields and tumour promotion in rats (Soffritti et al. 2015)

In the frame of the IARC assessment of extremely low frequency electromagnetic fields in 2001, animal studies related to breast cancer were discussed and evaluated, and the evidence rated as ‘inadequate’ according to the IARC Monographs Programme evaluation categories. Soffritti *et al.* (2016) investigated whether extremely low frequency electromagnetic fields have tumour-promoting effects. Sprague-Dawley rats (an outbred strain) were exposed to a magnetic field (50 Hz; 20 or 1000 μT) or sham-exposed beginning on the 12th day of pregnancy of the dams, and lasting until natural death of the offspring. At six weeks of age, the animals were additionally treated with an acute dose of 0.1 Gy of radioactive gamma radiation, which causes a dose-dependent increase in tumour development. Tumour incidence of radioactive exposure only was compared with combined exposure with radioactive radiation and the magnetic field. An increased tumour incidence regarding a) tumours of the mammary gland (both male and female animals), b) lymphomas/leukemia (male animals) and c) malignant schwannomas of the heart (male animals) was found in animals exposed to 1000 µT magnetic fields combined with radioactive gamma radiation. Regarding adenocarcinoma (malignant tumours) of the mammary gland, these effects were dose-dependent. Historical controls are available of the Sprague Dawley rats of this strain, and this information was used for the evaluation of tumour incidence. The experiments were not blinded. This is the first study with exposure starting at prenatal life and ending at natural death of the animals, combined exposure with ionizing radiation, and including both sexes. It is possible that the tumour promoting effects were not observed in previous experimental animal studies simply because the studies (or the exposure) ended at a specific earlier point in time.

Interplay between extremely low frequency magnetic fields and menadione (Kesari et al. 2016)

Using *in vitro* cultures of brain tumour cells from humans and rats, Kesari *et al.* (2016) investigated the postulated modulation of cellular effects of menadione (a provitamin) by weak extremely low frequency magnetic fields (50 Hz, 10 and 30 µT). They used multifactorial statistical methods for data analysis. In cell cultures, menadione causes an increase of the so-called micronuclei in a dose-dependent manner. They are isolated chromosomal segments outside of the cell nucleus, which are regarded as a product of DNA damage, which could either be caused by a menadione-dependent activation of enzymes that reduce nucleic acid, or by the development of free radicals in the mitochondria. First, cells were exposed to the magnetic field for 24 hours, and then treated with increasing concentrations of menadione for three hours. The authors found a weak but significant increase of micronuclei formation in the exposed human brain tumour cells, which appeared to be independent of free radical generation, and of the menadione concentration. Conversely, the exposed rat tumour cells showed more free radicals, but no significant increase of micronuclei. Considering older data with comparable experimental conditions (Luukkonen *et al.* 2011, 2014), a correlation between magnetic field intensity (0-100 µT) and increased formation of micronuclei and free radicals in mitochondria was found, yet, these effects seemed to be independent of menadione. This suggests that extremely low frequency magnetic fields do not modulate cellular response to menadione. A final assessment of the health relevance of these *in vitro* studies is difficult because of the not very pronounced and cell type specific effects. Nevertheless, they include some interesting fundamental observations.

2) Epidemiological studies

Do radiofrequency electromagnetic fields impair memory performance of adolescents? (Schoeni et al. 2015)

A prospective cohort study with 439 adolescents from Central Switzerland aimed at investigating whether memory performance in adolescents is affected by the cumulative EMF dose within one year from wireless device use, or by the wireless device use itself due to non-radiation related factors in that context. The study participants in the 7th and 8th grade performed a verbal and figural memory test using a standardized, computerized cognitive testing system, which was repeated after one year with 96% of the participants. Data on the use of wireless communication devices were collected from all study participants using questionnaires, and 53% of the participants consented to provide their operator recorded mobile phone use data. These data were incorporated in a dose calculation model (Roser *et al*. 2015) in order to assess the cumulatively absorbed EMF dose of the brain and the whole body in the period between the two memory performance tests. Sending text messages and playing computer games causes virtually no RF-EMF exposure, and were thus included in the analyses as negative exposure control variables for RF-EMF. The statistical analyses were adjusted for a range of confounders. It was found that the performance in a figural memory test decreased with increasing EMF dose. However, there was no apparent association regarding the number of sent text messages, or the time spent playing computer games. In general, verbal memory performance was not associated to EMF, but the results were depending on laterality: the subgroup of about 20% of study participants who also used their mobile phone on the left side of the head showed a tendency for decreased verbal memory performance. This is an interesting finding because the verbal memory involves mainly the left brain hemisphere. In general, the findings were consistent and similar regarding questionnaire and operator recorded data. The overall pattern of results suggests that rather the EMF dose than other aspects of mobile phone use affects the memory capacity of adolescents. This is the first longitudinal study with adolescents that includes brain dose calculations and objectively recorded mobile phone use data. A further strength of the study is the high proportion of participants that participated in the follow-up tests, and the consideration of objective data on mobile phone use. Most of the previous studies relied on self-reported exposure data of the study participants. Such estimations are unreliable. However, the sample size is relatively small. Currently, a follow-up study with 450 additional adolescents is ongoing.

Extremely low frequency magnetic field and breast cancer in men (Grundy et al. 2015)

Breast cancer in men is very rare, and only few studies have investigated a potential association with ELF-MF. A population-based case-control study in Canada investigated whether occupational exposure to ELF-MF increases the risk of breast cancer. The study included 115 cases registered in the Canadian National Enhanced Cancer Surveillance System between 1994 and 1998, and 570 controls. Occupational exposure to extremely low frequency magnetic fields was assessed by experts, and categorised into different exposure classes (<0.3, 0.3-0.6, ≥0.6 µT). Factors such as age, education, household income, marital status, body mass index and physical activity were considered in the analyses. None of the exposure variables was significantly associated to breast cancer risk. Breast cancer risk of persons with occupations involving exposure to extremely low frequency magnetic fields of at least 0.6 µT was non-significantly increased by a factor of 1.8 (95% CI: 0.82-3.95). In addition, a tendency was found for an association between breast cancer risk and the total time worked in an occupation with increased exposure (≥0.3 µT) to extremely low frequency magnetic fields (p=0.06). For persons that have worked in such an occupation for 30 years or longer, risk was increased by the factor 2.77 (95% CI: 0.98-7.82). Other exposure features such as age during first exposure or cumulative magnetic field exposure did not show any consistent correlations. Although this is one of the largest studies on this topic, the number of breast cancer cases is small, and the statistical power thus limited. In order to make more statistically precise statements regarding a potential risk, it will be necessary to perform a meta-analysis with other studies related to breast cancer in men.

3) Human studies

Are there biomarkers for electrohypersensitivity? (Belpomme et al. 2015, Andrianome et al. 2016)

To date, no parameter has been found that can be used for characterising or diagnosing electromagnetic hypersensitivity (EHS). In order to evaluate potential diagnostic criteria or biomarkers, 1,216 patients suffering from EHS and/or multiple chemical sensitivity (MCS) have been clinically and biologically examined in a study launched in 2009 (Belpomme *et al.* 2015). Following a standardised approach, the patients were thoroughly examined in a systematic manner, and treated depending on the result. The initial clinical examination of each patient comprised a detailed anamnesis, a physical examination, medical imaging of the brain by MRI and/or CT, carotid echodoppler, measurement of a pulsometric index of the brain by computerized ultrasonic cerebral tomosphygmography (UCTS), and the analysis of a series of biomarkers by commercially available tests. Patients were included in the study if their symptoms could not be attributed to another disease, and if they did not suffer from chronic diseases such as diabetes, cancer, and/or neurodegenerative or psychiatric diseases. 727 of the 839 cases that have been analysed so far fulfilled these inclusion criteria. The results of the biomarker tests were compared with the reference values provided by the test manufacturers. Depending on the test, between 6% and 55% were outside of the specified normal range. The authors thus conclude that EHS and MCS can be objectively characterised with simple tests. Furthermore, they suspect that both, EHS and MCS, show a similar pathologic mechanism, because the proportion of people outside normal range was similar in both groups.

Based on the data presented, the conclusions of the authors cannot be supported. The main limitation of the study is the lack of a control group, and the lack of a detailed discussion of the sensitivity, specificity, referencing and reproducibility of the applied tests. It is thus striking that for virtually all investigated biomarkers, the majority of examined persons was in the specified normal range. With all uncertainty regarding the informative value of that normal range, it is thus obvious that the specificity of these laboratory analyses is not sufficient in order to be able to use them as diagnostic criteria. Moreover, the consistently decreased melatonin levels are faced with the reservation that determining melatonin is very difficult, as it strongly reacts on outside influences such as light, lifestyle habits, diet and medication. In this case, as well as for all other biomarkers with a diurnal secretion profile, a single measurement is not informative and rather a 24-h profile is required, ideally over several days. The procedure for measuring the pulsometric index is not established, and only the result of a single case has been graphically presented. Therefore, the informative value of this method cannot be evaluated. The way in which data are presented in this publication does not contribute to a better understanding of EHS and MCS.

In another study (Andrianome *et al.* 2016), the self-reported sleep quality as well as the melatonin levels in saliva and urine of 30 EHS individuals was compared with 25 non-EHS individuals matched for gender, age and body mass index. The 30 EHS individuals were recruited from a previous survey on the topic. The 24-hour melatonin concentration was determined based on the urine collected separately during night and day, and the profile over the day based on 12 saliva samples collected at several time points. The subjective sleep quality in the EHS group was significantly lower than in the control group. The melatonin concentration in urine and saliva did not differ between the two groups, neither during night-time nor during daytime. A strength of this study is the inclusion of a control group. Only the comparison with a control group allows the identification of potential differences between EHS and non-EHS individuals. The sample size was relatively small, and, as is to be expected, the melatonin concentration varied considerably between individuals and across the day. Therefore, only rather large differences would have made it possible to revealing a statistically significant effect in this study. Potential confounders such as light exposure, sleeping times or chronotype have not been taken into account, and the recruitment of the control group is not described. It needs to be emphasised that neither of the two studies (Belpomme *et al.* 2015, Andrianome *et al.* 2016) has collected or analysed any data regarding EMF exposure of the study participants. For this reason, none of the studies is suitable for investigating an impact of EMF in everyday life on biomarkers. The aim of the two studies was rather to obtain specific, objective biomarkers for self-reported EHS. The findings of the first study (Belpomme *et al.* 2015) are not informative in this respect, and the second study (Andrianome *et al.* 2016) did not find a difference in melatonin levels between EHS individuals and the control group.

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References

Andrianome S, Hugueville L, de Seze R, Hanot-Roy M, Blazy K, Gamez C, Selmaoui B (2016): **Disturbed sleep in individuals with Idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF): Melatonin assessment as a biological marker.** Bioelectromagnetics. 2016 Mar 10. http://www.ncbi.nlm.nih.gov/pubmed/26969907

Belpomme D, Campagnac C, Irigaray P (2015): **Reliable disease biomarkers characterizing and identifying electrohypersensitivity and multiple chemical sensitivity as two etiopathogenic aspects of a unique pathological disorder.** Rev Environ Health. 2015 Dec 1;30(4):251-71. http://www.ncbi.nlm.nih.gov/pubmed/26613326

Grundy A, Harris SA, Demers PA, Johnson KC, Agnew DA; Canadian Cancer Registries Epidemiology Research Group, Villeneuve PJ (2016): **Occupational exposure to magnetic fields and breast cancer among Canadian men.** Cancer Med. 2016 Jan 21. http://www.ncbi.nlm.nih.gov/pubmed/26792203

Kesari KK, Juutilainen J, Luukkonen J, Naarala J (2016): **Induction of micronuclei and superoxide production in neuroblastoma and glioma cell lines exposed to weak 50 Hz magnetic fields.** J R Soc Interface. 2016 Jan;13(114). http://www.ncbi.nlm.nih.gov/pubmed/26791000

Luukkonen J, Liimatainen A, Höytö A, Juutilainen J, Naarala J (2011): **Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells.** PLoS One. 2011 Mar 23;6(3):e18021. http://www.ncbi.nlm.nih.gov/pubmed/21448285

Luukkonen J, Liimatainen A, Juutilainen J, Naarala J (2014): **Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells.** Mutat Res. 2014 Feb;760:33-41. Epub 2013 Dec 26. http://www.ncbi.nlm.nih.gov/pubmed/24374227

Megha K, Deshmukh PS, Banerjee BD, Tripathi AK, Ahmed R, Abegaonkar MP (2015): **Low intensity microwave radiation induced oxidative stress, inflammatory response and DNA damage in rat brain.** Neurotoxicology. 2015 Oct 25;51:158-165. http://www.ncbi.nlm.nih.gov/pubmed/26511840

Roser K, Schoeni A, Bürgi A, Röösli M (2015): **Development of an RF-EMF Exposure Surrogate for Epidemiologic Research.** Int J Environ Res Public Health. 2015 May 22;12(5):5634-56. http://www.ncbi.nlm.nih.gov/pubmed/26006132

Schoeni A, Roser K, Röösli M (2015): **Memory performance, wireless communication and exposure to radiofrequency electromagnetic fields: A prospective cohort study in adolescents.** Environ Int. 2015 Dec;85:343-51. Epub 2015 Oct 30. http://www.ncbi.nlm.nih.gov/pubmed/26474271

Soffritti M, Tibaldi E, Padovani M, Hoel DG, Giuliani L, Bua L, Lauriola M, Falcioni L, Manservigi M, Manservisi F, Panzacchi S, Belpoggi F (2016): **Life-span exposure to sinusoidal-50 Hz magnetic field and acute low-dose γ radiation induce carcinogenic effects in Sprague-Dawley rats.** Int J Radiat Biol. 2016 Feb 19:1-13. http://www.ncbi.nlm.nih.gov/pubmed/26894944

Weitere Informationen und Hintergründe zur beratenden Expertengruppe nicht-ionisierende Strahlung (BERENIS) sowie eine Übersicht der verwendeten Abkürzungen finden Sie auf http://www.bafu.admin.ch/elektrosmog/13893/15174/16478/index.html?lang=en

Link to list of abbreviations (pdf)