Summaries and assessments of selected studies

In the period from mid of January to end of April 2018, 92 new publications have been identified, and seven 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 this issue, among others, an animal study from Italy ("Ramazzini study", Falcioni et al. 2018) is discussed in connection with an animal study from the USA ("NTP study", NTP 2018a, 2018b, 2018c, 2018d, 2018e; Wyde et al. 2016, 2018a, 2018b) that was already partially discussed in Newsletter 07/2016. While this newsletter only contains a short summary and evaluation by BERENIS, a detailed review and evaluation of the NTP and Ramazzini studies is published in a Special Issue (November 2018).

1) Experimental animal and cell studies

Evaluation of two new cancer studies investigating lifetime radiofrequency exposure of mice and rats (Falcioni et al. 2018; NTP 2018a, 2018b, 2018c, 2018d, 2018e; Wyde et al. 2018a, 2018b)

Recently, the results of two comprehensive large-scale studies have been published. Both studies were performed with laboratory rats and mice roaming freely in cages to investigate the carcinogenic potential of lifetime exposure to radiofrequency electromagnetic fields (RF EMF). The focus of the study of the "U.S. National Toxicology Program" (NTP) was exposure associated with mobile phones (NTP 2018a, 2018b, 2018c, 2018d, 2018e; Wyde *et al.* 2018a, 2018b), while the study of the Ramazzini Institute in Italy focused on the far field of sources, i.e. exposure to mobile phone base stations or mobile phones of bystanders (Falcioni *et al.* 2018). In the following, the studies are referred to as "NTP study" and "Ramazzini Study", respectively.

The NTP and Ramazzini studies are the most comprehensive animal studies with regard to cancer and exposure to mobile phone and base station signals that have been conducted to date. The scientific quality and standard of laboratory techniques are high, especially in the NTP study. Compared to earlier studies with laboratory animals that evaluated carcinogenicity or co-carcinogenicity of RF EMF, these studies are novel in that the animals were exposed roaming freely in their cages instead of being placed in narrow tubes for exposure. This reduced the stress caused by confinement and allowed for longer daily exposures.

The results of these two animal studies are of great scientific relevance and importance for health policy because according to the International Agency for Research on Cancer (IARC), positive results from animal studies with lifetime exposure are very important with regard to the classification of cancer risk of an agent, together with data from epidemiological and mechanistic studies. Based on the observed evidence regarding a correlation between mobile phone use and gliomas as well as acoustic neuroma, the latter data led to the IARC classification of mobile phone radiation as 'possibly carcinogenic' (group 2B) in 2011. The IARC classifies the risk that an agent or environmental toxin causes cancer in humans into five groups, namely 'probably not carcinogenic' (group 4), 'not classifiable' (3), 'possibly carcinogenic' (2B), 'probably carcinogenic' (2A) or 'carcinogenic' (1).

Despite the methodological differences, both new animal studies showed relatively consistent results in schwannomas and gliomas, as well as a dose-dependent trend to an increase in the carcinogenicity of these tumors. The NTP study used high whole-body doses (SAR – specific absorption rates) as compared to the regulatory limits for whole-body exposure recommended by the International

Commission on Non-Ionizing Radiation (ICNIRP). For the general public, this limit is 0.08 W/kg, with Switzerland additionally having introduced lower precautionary limits. The question thus arises of how transferable the NTP study results are to real-life exposure of the public, considering that mobile phone use exposes only parts of the body to EMF levels comparable to the ones applied to the whole animal by the NTP study. First, it is common practice in toxicology to study higher doses to evaluate possible hazards of an agent. Second, the NTP study found an increase in carcinogenicity also for GSM and CDMA exposure conditions. Since the findings are similar for both types of exposure, they indicate that the modulation of the signals does not seem to be relevant. Third, mobile phone use can cause local SAR values up to 2 W/kg, averaged over a cube of 21 mm side length in the closest proximity of the phone (e.g., at the ear, cheeks, hand, pocket locations, etc.). Thus, the results of the NTP study are mostly relevant for the exposure situation when using a mobile phone close to the body. In contrast, the Ramazzini study observed carcinogenicity at levels as high as the environmental exposure limits, with no statistically significant effect at lower doses. However, a dose-dependent trend was found for malignant heart schwannomas, which is consistent with the findings of the NTP study. This may indicate that the non-significant increase in case numbers at lower exposure levels represents a true effect that has not reached statistical significance due to the given sample size.

In summary, BERENIS supports a precautionary approach for regulating RF EMF based on the findings and their evaluation. A full risk assessment analysis taking into account all available studies (animal studies and epidemiological studies) is necessary to assess whether the current standards should be changed.

➔ ***Note: A detailed evaluation of the NTP study and the Ramazzini study can be found in a special issue of the newsletter (November 2018)***

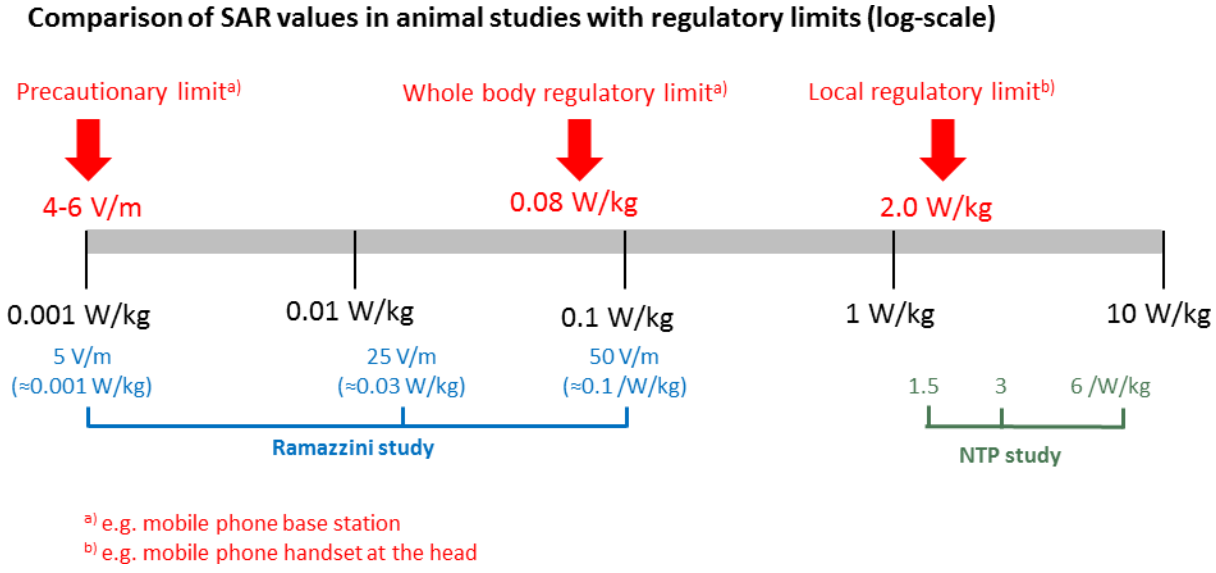

Figure 1: Comparison of SAR values in animal studies with regulatory limits (logarithmic scale)

Extremely low frequency magnetic fields and tumor development in rats (Bua et al. *2018)*

Bua et al. (2018) conducted another large animal study at the Ramazzini Institute. They investigated the influence of extremely low frequency magnetic fields (ELF MF) on tumor development in Sprague-Dawley rats, with more than 500 animals per group. In the present study, male and female rats were exposed to a magnetic field (50 Hz; 2, 20, 100 or 1000 µT (regulatory limit in Switzerland: 100 µT), continuous or intermittent (30 minutes on / 20 minutes off, 19 hours/day). The exposure started in the womb before the rats were born, and continued until their natural death. Survival time and body weight were comparable to the control animals of the same sex. Tumor incidence and the number of malignant and benign tumors were comparable in all groups. The authors concluded that exposure to a continuous or intermittent magnetic field (50 Hz) alone does not pose a significant risk for increased development of tumors in this rat model.

Group sizes were rather large in this study, which increases the statistical power and would have allowed for discovering trends even in tumors that are rather rare, such as C-cell carcinomas (tumors of the thyroid gland). The tumor incidence at the end of the animals' life was 35-38%, allowing for detecting differences in tumor incidence (higher/lower). Two pathologists analyzed the organs or tissue sections. For the histopathological analyses, a blinded design was used. Interestingly, in two recently published studies from the same institute a significantly higher incidence of malignant tumors of the mammary gland, C-cell carcinomas, heart schwannomas, and tumors of the blood-lymphoreticular system had been found in the highest dose group (1000 µT) (Soffritti *et al.* 2016a, Soffritti *et al.* 2016b, see newsletters 6 & 8). However, in these studies the magnetic field exposure was combined with low doses of formaldehyde or gamma radiation that do not cause tumors when applied alone. This implies that the combined effect is stronger than the individual effect of harmful substances, a phenomenon that has already been observed previously (IARC 2002). In everyday life, one is exposed to a variety of other environmental factors that may or may not contribute to harmful effects including carcinogenesis. This justifies a precautionary approach to limiting the individual agents.

Extremely low frequency magnetic fields enhance the effect of leukemia treatment (Provenzano et al. *2018) and accelerate wound healing (Patruno* et al. *2018)*

The *in vitro* studies by Provenzano *et al.* (2018) and by Patruno *et al.* (2018) dealt with potential therapeutic applications of ELF MF. Although such applications are not in the primary focus of BERENIS, they are discussed in this newsletter as the investigations are of good scientific quality and point to mechanisms of action that could be relevant for risk assessment when generalized. Apparently, signaling pathways responding to extra-cellular changes are involved. The "extracellular signal-regulated kinase" (ERK) plays a central role in the cellular response to systemic changes and environmental influences. Fundamental cell biological processes such as differentiation are regulated by ERK, however, altered activities of this signaling pathway are also characteristic for certain aetiopathologies. Furthermore, studies investigating effects of electromagnetic fields on cell health have frequently observed an altered activity of this signaling molecule. In this context, the results are of interest from a therapeutic point of view, and can provide important clues about the interaction of EMF with cellular physiology.

Provenzano *et al.* (2018) investigated the effect of a continuous ELF MF (50 Hz) on the treatment success in a specific type of leukemia (acute promyelocytic leukemia, APL). This type of leukemia is characterized by over-proliferation of an intermediate cell type of the blood differentiation due to a blockage in the promyelocytic stage. It can be treated by a vitamin-A-acid (all-trans-retinoic acid, ATRA), which promotes the differentiation of the leukemia cells and thus lead to a reduction of the

pathological cell division. While the exposure of leukemia cells with the ELF MF alone (0.5, 1, 2 mT) did not affect proliferation, the authors observed that the anti-proliferative effect of ATRA was increased by the ELF MF in a dose-dependent manner. Marker analyses showed that this is due to more efficient differentiation into neutrophilic granulocytes and not cell death. Furthermore, the authors studied the cause of this anti-proliferative effect and found that the combination of ELF MF and ATRA treatment resulted in an increase in reactive oxygen species (ROS), which was barely detectable for ATRA alone. In fact, they also found that neutralizing ROS abolishes the ELF MF effect on ATRA-dependent cell proliferation and differentiation. The authors also investigated the effect of ELF MF on signal proteins ERK1/2 and NFkappaB and found that ELF MF exposure led to increased ATRA-induced activation of ERK1/2. However, it remains unclear how these two observations are related. Interestingly, the effect of ELF MF on the efficiency of differentiation only seems to exist after ATRA treatment, but not when the leukemia cells were differentiated into monocytes. Since retinoic acid is an important factor in embryonic and especially neuronal development, the mechanistic findings of this study may well be considered for health risk assessment.

Wound healing is a complex multiphasic process that requires coordinated activation of a number of different cell types (immune cells, fibroblasts, and keratinocytes of the skin). The initial inflammatory response triggers the activation and proliferation of stem cells, which by means of migration and differentiation eventually leads to wound closure and renewal of the skin structure. The *in vitro* study by Patruno *et al.* (2018) investigated the effect of an ELF MF (50 Hz, 1 mT) on the migration of keratinocytes. They found that a scratch in a two-dimensional keratinocyte layer closed significantly faster while under ELF MF exposure compared to sham exposure, which was solely due to cell migration and not cell division. Moreover, an increased activation of the ERK1/2 and Akt signaling pathways was reported, which was most pronounced one hour after the start of exposure. Blocking these signaling pathways reduced the accelerated closure of the scratch under ELF MF exposure as wells as the expression and activity of metalloproteases. These proteins are located on the cell membranes and are able to, for example, degrade collagen and thus release cells from the cellular network promoting the accelerated migration. The authors postulate that this effect on cell migration by the exposure is due to altered profiles of pro- and anti-inflammatory cytokines[1]. In addition to the therapeutic aspects, the study is also noteworthy because of the observed influence of ELF MF exposure on the central signaling pathways, and the quite plausible description of molecular processes leading to the observed migration effect.

2) Reports

ANSES report on electromagnetic hypersensitivity (EHS) (ANSES 2018)

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) has published a comprehensive report on electromagnetic hypersensitivity (EHS)[2]. The aim of the expertise was to understand and characterize EHS in its complexity, and to investigate causes and pathogenesis. The report considered related scientific literature published from April 2009 to July 2016 and some older publications. Earlier literature had been discussed in previous reports focusing on “electromagnetic fields”. For a better understanding of the disease pattern, 64 letters written to ANSES by persons affected by EHS were analyzed in addition to the scientific literature, and interviews were conducted (with doctors treating EHS patients, with associations of EHS-affected persons, and with participants

[1] small proteins that promote or counteract inflammation
[2] also referred to as “idiopathic environmental intolerance attributed to electromagnetic fields” (IEI-EMF)

of a roundtable discussion). In a last step, a public consultation was held in order to supplement the report.

The 16-member expert group found that in general, the related literature was subject to major methodological deficits. In particular, this referred to recruitment processes that were highly heterogeneous, given that objective and generally accepted criteria for the characterization of EHS patients did not exist. The investigation of symptoms and sources was considered being heterogeneous as well. Accordingly, prevalence data were evaluated with reservations, with recent literature pointing at a prevalence of roughly five percent (and not increasing). In some studies, people with a relatively low threshold of perception for low-frequency fields were found, however, overall the current state of research did not show a robust correlation between EMF exposure and EHS symptoms. The fact that experimental provocation tests had negative results could mean that there is in fact no correlation, but it is also possible that methodological weaknesses of the studies - especially with regard to recruitment - are the reason why a true association might be unrecognized. It was also possible that the effects manifested themselves only under certain conditions or exposure situations that have not yet been understood. A total of 18 hypotheses on EHS pathogenesis were investigated. Nocebo effects that are well described in the scientific literature promoted the persistence of complaints, but did not rule out EMF as a trigger. The scientific evidence suggested that EHS patients have a decreased level of well-being, and are more depressed and anxious than non-EHS persons, although it is typical that chronic and rare diseases are accompanied by increased anxiety and depression. The prevalence of pathological personalities or psychiatric disorders was not increased. There were links to MCS[3], fibromyalgia, migraine and tinnitus. The hypothesis that EHS patients show specific traits in the functional state of the autonomic nervous system could not be evaluated due to methodological deficits. The expert group evaluated hypotheses of a disorder of the blood-brain barrier as well as disorders in the neurotransmitter production as unconvincing. The hypothesis that EHS could be classified as a type of migraine, based on successful treatment trials with migraine remedies, was evaluated as convincing. More research was needed in this respect. Potential connections to fibromyalgia should be looked into more closely, particularly skin symptoms associated to EHS, as these may be related to an anomaly of small peripheral skin nerves associated with fibromyalgia. Furthermore, in the view of the expert group, the hypothesis of circadian rhythm disorders as a possible explanation for frequent complains of sleep disturbances as well as the hypothesis of hypersensitivity as a personal trait should be examined in more depth.

The working group recommends creating counseling services with medical supervision, developing guidelines for medical practitioners, and financing continuous research for provocation studies with improved recruitment, realistic and individualized exposure, and different exposure protocols. In addition, health measurements should be supplemented with objective methods such as polysomnographies and biomarkers.

Regarding support structures outside of France, the report highlights the environmental medical counseling service of a group of medical doctors in Switzerland (Ärztinnen und Ärzte für Umweltschutz, www.aefu.ch).

[3] Multiple Chemical Sensitivity

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

List of abbreviations (pdf)