Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on "*In vivo* genotoxicity of high-intensity intermediate frequency magnetic fields in somatic cells and germ cells" by Ohtani et al. in Journal of Radiation Research (2022)

Category [intermediate frequency, animal study]

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Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

There has been a rapid expansion of wireless power transfer (WPT) abilities in mobile phone chargers, stationary charging electric vehicles and road-powered electric vehicles. One of its components are intermediate frequency magnetic fields (IF-MF, 300 Hz - 10 MHz), specifically the 20 kHz - 150 kHz frequency range that is relevant for charging electric vehicles [2]. Owing to the increasing proliferation of technologies using WPT, there is a need to accumulate science-based evidence about potential health effects. To date, few studies have investigated health risks of IF-MF, the majority of them found no adverse effects [3].

2 Results and conclusions from the authors perspective

To investigate whether short-term exposure to high-intensity IF-MF could lead to putative health effects, genotoxic effects were investigated in somatic and germ cells in mice exposed *in vivo*.

Male mice were divided into five different groups: sham-exposed, IF-MF-exposed, X-irradiated (as positive control for the micronucleus and the pig-a mutation assay), negative control (untreated) and ENU-administered (N-ethyl-N-nitrosourea, as positive control for the gpt mutation assay). The IF-MF-exposed group was exposed to 82.3 kHz with an average electric field induced throughout the mouse body of 87 V/m (3.8 times the basic restriction for occupational exposure [4]). The exposure duration lasted for 1 day or 10 consecutive days, which was acquired 30 times per day with a total exposure time of 90 s.

DNA damages were investigated using the micronucleus (MN) test. Micronuclei are mainly acentric chromosomes that arise as a result of DNA-damaging events. For this purpose, blood samples were taken before exposure and on days 0 (immediately after exposure), 2, 3, 6, 10 and 14 after exposure. Further samples were taken before exposure and at days 2, 7 and 14 after exposure for additional mutation assays. For positive controls, mice were either irradiated with 0.5 or 3 Gy of ionizing radiation or administered with the alkylating agent N-ethyl-N-nitrosourea (ENU) once a day for 5 consecutive days. For the analysis, hematopoietic cells (reticulocytes and erythrocytes) were extracted from blood samples, immunostained and analyzed by fluorescence-activated cell-sorting (FACS). In germ cells (spermatids, spermatocytes, spermatogonia and sperm), MN were analyzed microscopically in 4',6-Diamido-2-phenylindol (DAPI)-stained testis samples after 10 days of exposure.

To detect mutations, two different mutation assays were used: the pig-a assay and the gpt assay. Briefly, the pig-a assay is used to detect mutations in the endogenous reporter gene phosphatidyl inositoglucan class a (pig-a), the gpt assay is used to determine mutations in the guanine-phosphoribosyltransferase (gpt) gene, which is carried by certain transgenic mouse strains. For these mutational assays, either hematopoietic cells were labeled with antibodies and analyzed by FACS (pig-a assay) or DNA was extracted from the liver, spleen, bone marrow and testis and the mutation frequency of the gpt gene was analyzed (gpt assay).

The frequency of MN in hematopoietic cells was not affected by IF-MF exposure: no statistically significant differences between the IF-MF-exposed group (n= 6) and the sham control group (n= 6) were found. Also, no significant differences were found between the IF-MF-exposed (n= 6) and the sham control group (n= 6) of hematopoietic cells in the pig-a mutation assay, except for day 2, where the mutation frequency in mature erythrocytes was even lower than in the sham-exposed group.

In germ cells and spermatids the MN frequency was not significantly different between the IF-MF-exposed (n=5) and the sham control group (n= 5). The mutation frequency analyzed by the gpt assay showed no statistically significant differences between the IF-MF-exposed (n= 6) and the sham-exposed group (n= 6).

Based on the results of this study, short-term high-intensity IF-MF did not lead to any genotoxic effects in somatic and germ cells.

3 Comments by the BfS

The research question addressed in this study is of scientific interest and relevant for radiation protection. Generally, the authors used adequate methods to answer the underlying question and it is worthwhile to note that positive and negative controls are used in all experiments.

Limitations remain in view of the small number of animals used and the dosimetry: the information on exposure lacks many details and thus, the exposure is difficult to assess and could not be reproduced. The study refers to a previous study [5] that does not mention the external field strength and the cited studies [5, 6] used slightly different parameters. However, the exposure scenario can be roughly transferred so that the external field strength was probably in the tens of mT range. From the data given it is not possible to determine how the electric field strength was distributed in the mouse body and what range of internal field strengths were reached. Furthermore, it is noticeable that the sham exposure was not performed with antiparallel wound coils, therefore a residual risk remains that the experimental conditions were not identical.

The measurement of MN frequency in hematopoietic cells by FACS analysis is less accurate than an analysis under the microscope. MN mostly consist of acentric- or lagging chromosomes, which can best be observed microscopically during cellular division under the microscope, in particular after cytochalasin b treatment, which inhibits cytoplasmatic division, but nuclear division during cytokinesis. For this experimental setup on the other hand, with this many different treatments and time points, the high-throughput FACS analysis is a good compromise.

For the MN-analysis of different germ cells, the nuclei were stained with DAPI and the samples were analysed microscopically. The method used could be more accurate – without using cytochalasin b there is a risk that nuclear fragments from other (e.g. apoptotic) cells might be confound with micronuclei.

Mutations in hematopoietic cells were also studied by means of the pig-a assay, which is considered a reliable tool for quantifying *in vivo* and *in vitro* mutational events in mammalian cells. The authors themselves stated that in this study the pig-a mutation frequency showed a considerable variability. The authors attribute this to the high baseline variation in mature erythrocytes. Indeed, the variability and scattering are very high; much higher than in the previous study [7] and in other studies [8]. On the other hand, a significant increase in the positive control group was found, so the general functionality of the pig-a assay was considered. Overall, the results are reliable, but compromised by the high variability and scattering of the results.

To detect mutations in liver, spleen, bone marrow and testis the authors used the gpt assay, that only works in transgenic mice that were used specifically for this assay. It is a generally accepted method for identifying and characterizing genotoxic hazards and for determining the mode of action of carcinogens. This mutation assay is considered reliable, although clastogenic events are less likely to be detected [9]; so an additional analysis of chromosomal aberrations in liver, spleen, bone marrow and testis would have been advantageous to support the mutation data from these tissues.

This study fulfills many criteria of good scientific practice. Only the exposure assessment is not described in detail so that it is unclear, which field strength was actually applied and a consequent blinding of the research personnel is missing (or at least only mentioned for the MN test in germ cells). The study investigated genotoxicity with several different methods and these are well chosen to investigate the research question. Overall, the study provides an important contribution to the risk assessment of IF-MF.

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