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**EHAB MUSTAFA**

# *IN VITRO* RESPONSES TO ELECTROMAGNETIC FIELDS: GENOTOXICITY, OXIDATIVE STRESS, AND CIRCADIAN RHYTHM

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*Ehab Mustafa*

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# ABSTRACT

Emerging technologies resulted in ubiquitous exposure to electromagnetic fields (EMFs). Humans are continuously exposed to radiofrequency (RF) radiation and extremely low frequency (ELF) magnetic fields (MFs). RF radiation is emitted from several wireless applications, while ELF MFs accompany electricity generation, transmission, and use. Over the years, the question has been raised about possible health effects of exposure to EMFs. In 2002, the International Agency for Research on Cancer (IARC) classified ELF MFs as possibly carcinogenic to humans based on the epidemiological findings suggesting an association between childhood leukemia and residential exposure to weak MFs from power lines. Further, in 2013, IARC classified RF radiation as possibly carcinogenic to humans based on epidemiological studies indicating an increased risk of glioma and acoustic neuroma among mobile phone users. However, in the context of IARC classification, the evidence for carcinogenic effects from animal and in vitro studies was inadequate. Therefore, establishing plausible biophysical mechanisms that explain the possible association between cancer and exposure to ELF MFs and RF radiation remains a high research priority.

At an in vitro level, the present study aimed to explore possible effects of RF radiation and ELF MFs on specific cancer-related phenomena, namely: genotoxicity, genomic instability, oxidative stress, and circadian rhythm. RF experiments were conducted using rat primary astrocytes to test whether RF radiation has genotoxic or co-genotoxic effects and whether it could induce or enhance genomic instability. ELF MF experiments were conducted using mouse hematopoietic FDC-P1 cells and human SH-SY5Y neuroblastoma cells. In ELF MF experiments, possible genotoxic and co-genotoxic effects were investigated. In addition, it was investigated whether ELF MFs could modify the repair rate of chemically-induced DNA damage or alter the transcription of DNA damage signaling-related genes. Furthermore, it was examined whether ELF MFs could affect the level of reactive oxygen species (ROS) or change the transcription of oxidative stress-related genes. To evaluate possible effects on the circadian rhythm, it was tested whether ELF MFs could alter the transcription of circadian rhythm-related genes, including *CRY1* and *CRY2*; the genes encoding the magnetosensitive flavoproteins, cryptochromes.

No genotoxic effects were found from 872 MHz RF exposure at SAR 0.6 or 6 W/kg. The results did not consistently support co-genotoxic effects from RF exposure. RF radiation was not shown to induce or enhance genomic instability.

Sole exposure to 50 or 60 Hz, ELF MFs at 100 or 200 μT, did not affect DNA damage level. The results showed contrasting responses to ELF MF exposure on chemically-induced DNA damage repair rate. While 24-h exposure to 50 Hz MFs decreased bleomycin-induced DNA damage repair rate in FDC-P1 cells, no such effect was seen in SH-SY5Y neuroblastoma cells exposed to 50 or 60 Hz MFs at 100 μT for the same exposure duration. ELF MFs effects on the DNA damage-signaling gene transcription were sporadic and generally small. Exposing SH-SY5Y neuroblastoma cells to 50 or 60 Hz MFs at 100 μT resulted in a small

increase in the transcription of ROS-inducible (antioxidant) genes, which was followed by a slight decrease in menadione-induced ROS levels at several time points after the exposure. No evidence was found that ELF MFs affected the transcription of core circadian rhythm-related genes, including *CRY1* and *CRY2*. However, a noteworthy systematic change was observed in the expression of circadian rhythm-related genes in FDC-P1 cells after 12- and 24-h exposures to 200 μT, 50 Hz MFs.

In conclusion, the present study provided new insights that help increase understanding of possible EMF carcinogenic effects. This present study results showed no evidence for genotoxic or co-genotoxic effects from RF exposure and reported that RF radiation did not induce or enhance genomic instability. The observed effects of ELF MF exposure on the assayed endpoints were generally small, suggesting that ELF MFs effects are on cellular signaling rather than inducing oxidative stress or DNA damage.

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Kuopio, February 2022
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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AM | Amplitude modulation |
| CRY | Cryptochrome |
| CW | Continuous wave |
| EC | European commission |
| ELF | Extremely low frequency |
| EMF | Electromagnetic field |
| FM | Frequency modulation |
| g | Gram (unit of weight) |
| GSM | Global System for Mobile Communications |
| ICNIRP | International Commission on Non-Ionizing Radiation Protection |
| IF | Intermediate frequency |
| IGI | Induced genomic instability |
| IR | Infrared |
| LTE | Long-term Evolution |
| m | Meter (unit of length) |
| MF | Magnetic field |
| NTP | National Toxicology Program of the United States of America |
| RF | Radiofrequency |
| RPM | Radical pair mechanism |
| SAR | Specific absorption rate |
| SCENIHR | Scientific Committee on Emerging and Newly Identified Health Risks |
| T | Tesla (unit of magnetic flux density) |
| UMTS | Universal Mobile Telecommunications System |
| UV | Ultraviolet |
| W | Watt (unit of power) |
| WHO | World Health Organization |

# LIST OF ORIGINAL PUBLICATIONS

Chapter 2 Mikko Herrala., **Ehab Mustafa**, Jonne Naarala, and Jukka Juutilainen. (2018). Assessment of genotoxicity and genomic instability in rat primary astrocytes exposed to 872 MHz radiofrequency radiation and chemicals. *International journal of radiation biology*, 94(10), 883–889. https://doi.org/10.1080/09553002.2018.1450534

Chapter 3 **Ehab Mustafa**, Jukka Luukkonen, Jenny Makkonen, and Jonne Naarala. (2021). The duration of exposure to 50 Hz magnetic fields: Influence on circadian genes and DNA damage responses in murine hematopoietic FDC-P1 cells. *Mutation research – fundamental and molecular mechanisms of mutagenesis*, 823, 111756. https://doi.org/10.1016/j.mrfmmm.2021.111756

Chapter 4 **Ehab Mustafa**, Leonardo Makinistian, Jukka Luukkonen, Jukka Juutilainen, and Jonne Naarala. Do 50/60 Hz magnetic fields influence oxidative or DNA damage responses in human SH-SY5Y neuroblastoma cells? *International journal of radiation biology*, Online ahead of print. https://doi.org/10.1080/09553002.2022.2055803

# AUTHOR'S CONTRIBUTION

Chapter 2 Jukka Juutilainen, Jonne Naarala, and Mikko Herrala conceptualized and planned the study. **Ehab Mustafa** and Mikko Herrala conducted the experiments at the laboratory. **Ehab Mustafa** was responsible for the experiments that assayed possible immediate genotoxic/co-genotoxic effects (DNA damage and micronucleus frequency) of radiofrequency radiation at 0.6 W/kg. Mikko Herrala prepared the first draft of the study manuscript. All authors contributed to the subsequent manuscript revisions.

Chapter 3 **Ehab Mustafa**, Jukka Luukkonen, and Jonne Naarala conceptualized and planned the study. **Ehab Mustafa** conducted the experiments at the laboratory and prepared the first version of the study manuscript. Jenny Makkonen helped with using the gene expression facilities. All authors contributed to the subsequent manuscript revisions.

Chapter 4 **Ehab Mustafa**, Jukka Luukkonen, and Jonne Naarala conceptualized and planned the study. **Ehab Mustafa** conducted the experiments at the laboratory and prepared the first version of the study manuscript. All authors contributed to the subsequent manuscript revisions.

# CONTENTS

# Chapter 1.

## General introduction to electromagnetic fields and their related health effects

# 1 LITERATURE REVIEW

## 1.1 ELECTROMAGNETIC FIELDS

A stationary electric charge is surrounded by an electric field. When electric charges flow (in a current), a magnetic field is generated. In its simplest form, an electromagnetic field (EMF) is the combination of the electric field and the magnetic field that propagate in space perpendicular to each other at the speed of light. An EMF is primarily described by frequency and wavelength. Frequency is the number of waves that pass by a certain point in unit time. Wavelength is the distance between two successive identical points in the wave cycle. An equation determines the relationship between the frequency and wavelength: $V = f\lambda$; where V is the wave velocity, f is the frequency, and $\lambda$ is the wavelength. The wave velocity is constant (as long as the EMF does not pass to a different medium). Thus, any increase in frequency implies a decrease in wavelength. Therefore, wavelength and frequency are inversely proportional. In near fields (approximately within one-wavelength distance from the emitting source), electric and magnetic fields are separate. However, in far fields, electric and magnetic fields are coupled and referred to as electromagnetic radiation.

Electromagnetic radiation has a dualistic nature. It behaves as a wave, and it consists of photons. The photon energy is directly proportional to the frequency of the field. Depending on the photon energy, the electromagnetic spectrum (Fig. 1) is classically divided into ionizing radiation and non-ionizing radiation. Ionizing radiations such as X-rays and gamma rays have sufficient energy to ionize the matter by removing the tightly bound electrons from the atoms. On the other hand, non-ionizing radiation does not have enough energy to ionize the matter. The non-ionizing electromagnetic spectrum contains static, extremely low frequency (ELF), intermediate frequency (IF) electric and magnetic fields, radiofrequency (RF) radiation, infrared (IR) radiation, visible light (V), and ultraviolet (UV) A and B radiation. UVC radiation locates at the borderline between ionizing and non-ionizing radiation. Unlike other EMFs, the static electric and magnetic fields do not vary over time and, hence, they do not oscillate; thus, they are sometimes said to have a frequency of 0 Hz.

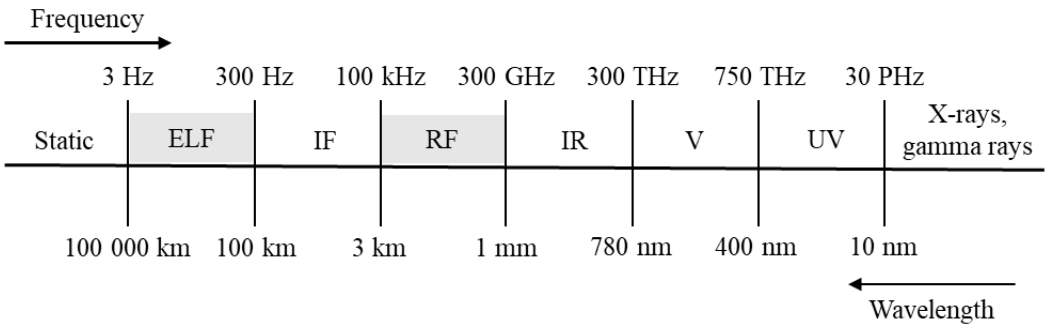


Figure 1. The electromagnetic spectrum. ELF: extremely low-frequency magnetic fields, IF: intermediate frequency magnetic fields, RF: radiofrequency radiation, IR: infrared radiation, V: visible light, UV: ultraviolet radiation. The frequency ranges of ELF magnetic fields and RF radiation (used in the present study) are highlighted in grey.

### 1.1.1 RF radiation

The term "RF radiation" is typically given to the EMFs in the band between 100 kHz and 300 GHz, including the microwave radiation that spans between 1 GHz and 300 GHz. RF radiation is utilized in various applications such as wireless communications, TV and radio broadcasting, radar surveillance, and microwave heating. The intensity of the RF radiation is expressed as power density in units of Watts per square meter ($W/m^2$). Specific absorption rate (SAR) describes how much energy is absorbed by the matter due to RF exposure. SAR is expressed in units of Watts per kilogram (W/kg). Human exposure to RF radiation has grown rapidly in the last few decades, mainly due to the high increase in mobile phone use. Besides, RF radiation is modulated to carry information. For example, frequency modulation (FM) and amplitude modulation (AM) are used in radio broadcasting. However, more complex modulations such as The Global System for Mobile Communications (GSM), Long-term Evolution (LTE), and The Universal Mobile Telecommunications System (UMTS) are utilized in mobile communications. Unmodulated RF radiation is referred to as continuous wave (CW). The basic restrictions for RF exposure issued by the European Council for whole-body exposure are 0.08 W/kg for the general public and 0.4 W/kg for occupational exposure, while the basic restrictions for local exposures (averaged over 10 g of tissue) are 2 W/kg (head and trunk) and 4 W/kg (limbs) for the general public, and 10 W/kg and 20 W/kg for occupational exposure (EC, 1991, 2004), respectively.

### 1.1.2 ELF magnetic fields

ELF magnetic fields (ELF MFs) are alternating fields with a frequency below 300 Hz. Natural and artificial ELF MFs exist. However, human exposure to ELF MFs is primarily associated with electricity generation, distribution, and use (mainly at 50 Hz and 60 Hz, commonly referred to as power-frequency MFs). The intensity of ELF MFs is expressed as magnetic flux density in units of Tesla (T). Humans are typically exposed to ELF MFs in household settings at a worldwide average intensity of ~0.21 μT from the domestic appliances and nearby power lines (SCENIHR, 2015). However, exposure to stronger intensities can occur in occupational settings, for example, working with welding machines or melting furnaces that use high currents. The reference levels recommended by the European Council for exposure to 50 Hz MFs are 100 μT for the general public and 500 μT for occupational exposure (EC, 1991, 2004); these reference levels were obtained from the basic restrictions by mathematical modeling.

## 1.2 GENOTOXICITY AND INDUCED GENOMIC INSTABILITY

Genotoxicity is defined as the ability of a chemical, physical, or biological agent to cause damage to the genome (Klaassen et al., 2013). Preservation of the genome is fundamental for all species. Thus, damage to the genome results in immediate responses, such as DNA repair machinery initiation, cell cycle arrest, cell cycle checkpoint activation, or apoptosis if the damage is severe. The failure of these responses may lead to mutations. Accumulation and persistence of mutations may eventually lead to cancer.

Induced genomic instability (IGI) refers to the *de novo* appearance of genomic abnormalities (e.g., chromosomal aberrations, micronuclei, or mutations) in the progeny of exposed cells several generations after exposure (Baverstock, 2000). IGI is a relatively new concept in cancer risk assessment, and its etiology remains poorly understood. However, it has been proposed that it originates from the accumulating mutations in DNA repair genes where various epigenetic mechanisms are involved (Negrini et al., 2010; Huumonen et al., 2014).

Although they are originally different, genotoxicity and IGI can be assayed with the same methods. The decisive factor is the chronological point at which the assay is done. Genotoxicity is assayed directly after exposure, while IGI is assayed several cell generations after exposure (Herrala et al., 2019). There are several methods to assay genotoxicity (for a review, see Corvi and Madia, 2017). Comet assay and

micronucleus assay are among the most frequently used methods. Comet assay can measure immediate DNA damage and DNA damage repair (Singh et al., 1988), while micronucleus assay quantifies micronuclei formation indicating chromosomal damage after the repair process (Fenech, 2000).

## 1.3 OXIDATIVE STRESS

Oxidative stress is the imbalance between free radical formation and the antioxidant defense system. Free radicals (most notably, reactive oxygen species, ROS) are by-products of normal cell metabolism. At a physiological concentration, ROS play a central role in cellular signaling. However, exposure to environmental factors (including radiation) usually results in increased ROS production. When ROS production exceeds the capacity of the antioxidant defense system, the cell enters to an oxidative stress status (Pizzino et al., 2017).

ROS are involved in different aspects of cancer. Excessive ROS production can cause direct damage to macromolecules, including DNA, which, if not repaired, could lead to mutations and, subsequently, cancer. Besides, ROS are vital in DNA damage signaling. ROS are found to regulate the transcription of DNA damage-recognition machinery and control the entry to different cell cycle phases where DNA damage is surveilled and repaired (Shackelford et al., 2000; Mori et al., 2017). In addition, abnormal ROS levels are thought to alter signaling and gene expression related to cell growth and thus cause continuous and uncontrolled proliferation (Valko et al., 2007).

Measurement of ROS production is a direct indicator of oxidative stress status. The fluorometry-based approach is one of the most used methods to assay ROS production. This approach involves the use of non-fluorescent probes that are oxidant-sensitive. In the presence of ROS, these probes are oxidized by a one-electron free radical mechanism producing fluorescent products that can be quantified spectrofluorimetrically (Zhang et al., 2018). Examples of other oxidative stress markers and assays include oxidative DNA damage, lipid peroxidation, expression of ROS-related genes, total antioxidant status, levels of glutathione and oxidized glutathione, and the activity of oxidative stress-related enzymes (e.g., catalase, superoxide dismutase, peroxidase, or glutathione reductase) (Olowe et al., 2020).

## 1.4 CIRCADIAN RHYTHM

Circadian rhythm is the internal autonomous oscillator that regulates physiological activities and allows the organism to adapt to the fluctuating environment around the ~24-h sleep/wake cycles (Xie et al., 2019). In mammals, the circadian rhythm compromises a central clock and a peripheral clock. The central clock locates in the hypothalamic suprachiasmatic nucleus in the brain and is solely affected by light perceived by the retina. Light receptors in the retina transform signals into nerve impulses, which are then transmitted to the hypothalamus. The peripheral clock is present in various tissues, including the kidney, heart, lung, and liver. In addition, the peripheral clock can respond to light and several nonlight environmental factors (Brainard et al., 2015).

At a molecular level, the circadian rhythm originates from the ~24-h oscillation in the products of eight core circadian clock genes: *BMAL1* (also known as *ARNTL*), *CLOCK*, *CRY1*, *CRY2*, *CSNK1ε*, *PER1*, *PER2*, and *PER3*. These genes are organized in a composite and autoregulated network (Fu and Lee, 2003). Circadian rhythm significantly contributes to regulating the cell cycle, DNA damage responses, and ROS signaling (Masri et al., 2013). Thus, disruption of the circadian rhythm is believed to play a substantial role in the evolution and progression of cancer (Li, 2019).

Different methods exist for assessing circadian rhythm (for a review, see Reid, 2019). Cortisol and melatonin are the traditional circadian markers. However, these are predominantly used in human and

animal studies. At *in vitro* level, assaying the expression of the core circadian clock genes is a widely used approach to assess the influence of exposures on the circadian system (Wittenbrink et al., 2018; Zhang et al., 2020).

## 1.5 HEALTH EFFECTS OF RF RADIATION, ELF MFs, AND THEIR RELATED MECHANISMS

Typical exposure to RF fields and ELF MFs occurs at low levels. Public health concerns primarily arise from the continuous and long-lasting exposure to these fields, in addition to the large fractions of the population that are exposed.

RF radiation

Tissue heating is the most well-established effect of strong RF fields. This effect arises from the ability of RF radiation to penetrate the body and deliver vibrational energy to biological molecules. A rise in body core temperature by more than approximately 1°C results in hyperthermia (excessive sweating, dyspnea, and heart arrhythmia), while body core temperature > 40 °C could lead to heatstroke (blood vessel dilatation, organ failure, and eventually death) (ICNIRP, 2020a). However, at exposure levels found in the everyday environment, much of the discussion is directed at the non-thermal effects of RF radiation. The non-thermal effects of weak RF fields are controversial, and to date, no mechanism has been proven to explain them (ICNIRP, 2020). Particularly, the RPM does not seem applicable with high-frequency MFs, including the frequency range where RF fields span (Sheppard et al., 2008; Hore and Mouritsen, 2016). It has been suggested that RF effects are dependent on signal modulation. However, in their review, Juutilainen et al. (2011b) did not find consistent evidence for such a suggestion.

Despite the lack of a known mechanism, IARC, in 2011, classified RF fields as possibly carcinogenic to humans, class 2B (Baan et al., 2011; IARC 2013). This classification was mainly based on limited epidemiological evidence suggesting an increased risk of glioma and acoustic neuroma among mobile phone users, in addition to limited evidence of carcinogenicity from animal studies. However, there was a minority opinion within the IARC Working Group that epidemiological evidence was inadequate (Baan et al., 2011). Furthermore, the Scientific Committee on Emerging and Newly Identified Health Risk (2015) concluded that epidemiological studies do not indicate increased risks for brain or head/neck cancers from exposure to mobile phone radiation (SCENIHR, 2015). Moreover, in their review of animal studies, Juutilainen et al. (2011a) indicated no evidence for carcinogenic effects from exposure to mobile phone radiation at levels relevant to human exposure.

Recently, two large and long-term animal carcinogenicity studies were performed within the United States National Toxicology Program (NTP, 2018a, b) and Italy's Ramazzini Institute (Falcioni et al., 2018). In these two studies, animals were exposed over their whole lives at intensities similar to or slightly higher than human exposure to RF radiation from mobile phones or base stations, demonstrating RF fields are carcinogenic. The International Commission on Non-Ionizing Radiation Protection (ICNIRP, 2020b) has critically evaluated these studies. However, despite several strengths, the ICNIRP evaluation inferred that substantial weaknesses (primarily, lack of blinding and difficulties in interpreting statistical analyses) precluded these studies from concluding carcinogenic effects.

Apart from cancer, investigating other adverse health effects from exposure to weak RF fields, such as effects on reproduction, development, immunity, and nervous system, has provided inadequate evidence (IARC, 2013; SCENIHR, 2015).

ELF MFs

High fields (with an intensity of several mT) can induce currents inside the body. These currents, if strong enough, can cause stimulation of nerves and muscle cells, leading to health hazards such as hoarseness, pain, dyspnea, heart arrhythmia, cardiac arrest, or even death (WHO, 2007). However, the biological effects of low fields (< 1 mT) are controversial, and the mechanisms behind them are poorly understood (ICNIRP, 2010).

In the two decades between 1979 and 1999, epidemiological studies rather consistently indicated an association between exposure to weak MFs and an increased risk of childhood leukemia. These studies were combined in two pooled analyses (Ahlbom et al., 2000 and Greenland et al., 2000), demonstrating the increased risk is associated with the residential exposure to 0.3 – 0.4 μT MFs from power lines. These epidemiological findings were the main reason that prompted the International Agency for Research on Cancer to classify ELF MFs as possibly carcinogenic to humans, class 2B (IARC, 2002). However, animal and cell studies provided only limited evidence for carcinogenic effects. Besides, it should be noted that pooled analyses of more recent epidemiolocal studies showed a decrease in effect or no association between MF and childhood leukemia (Amoon et al., 2018, 2021).

Several hypotheses have been suggested to explain the epidemiological findings of weak MFs (Zhadin and Barnes, 2005; Shaw et al., 2015; Binhi and Prato, 2017). Much of the discussion centers on the radical pair mechanism (RPM). The RPM is primarily based on the chemical reactions involving the formation of transit intermediates known as radical pairs. Radical reactions are found to be sensitive to MFs, as weak as those typically encountered in the environment (Steiner and Ulrich, 1989). According to the RPM, the effect of MFs on radical reactions increases the concentration of free radicals in low fields (fields with an intensity < 1 mT, low-field effect, LFE) and decreases it in high fields (Brocklehurst and McLauchlan, 1996; Timmel et al., 1998). Thus, within the framework of RPM, attention is directed to the LFE-increased levels of free radicals. That being so, this increase could result in radical-induced DNA damage, which, if not properly repaired, may eventually result in cancer.

Although RPM is one of the most plausible mechanisms explaining the epidemiological findings of weak MFs, the framework of this hypothesis remains challenging, particularly in the presence of relatively stronger (static) geomagnetic fields (25 – 65 μT) (Hore, 2019). Moreover, the increased level of free radicals in low fields seems to be small, momentary, and non-observable after being regulated by the cell defense systems (Markkanen et al., 2010; Juutilainen et al., 2018).

At a physiological concentration, free radicals are critical regulatory mediators in signaling processes within the cell. Thus, alteration in the level of these radicals, even if small and momentary, could result in significant consequences (Dröge, 2002), especially if this alteration occurred in organelles or molecules that are key components in the biological network, such as cryptochromes (CRYs) (Juutilainen et al., 2018). CRYs are a class of magnetosensitive flavoproteins found in different species. CRYs are shown, at least in birds, to be involved in orientation and navigation via sensing geomagnetic fields (Hore and Mouritsen, 2016; Xu et al., 2021). In mammals, CRYs are a key component in controlling the circadian rhythm, which is closely coupled to regulating DNA damage responses and ROS signaling (Michael et al., 2017). Juutilainen et al. (2018) have proposed a hypothesis for environmental magnetocarcinogenesis. This hypothesis implies that the primary interaction between weak MFs and radical reactions in the CRYs could result in impaired DNA damage responses, compromised ROS signaling, genomic instability, and finally, cancer.

Apart from cancer, other adverse health effects from exposure to weak ELF MFs have been investigated, such as neurological disorders and reproductive hazards. However, these investigations provided only limited evidence for such effects. (Juutilainen, 2003, 2005; Vergara et al., 2013; Lewis et al., 2016; Jalilian et al., 2018).

## 1.6 SUMMARY OF GENOTOXICITY FINDINGS LINKED TO RF RADIATION

Studies exploring possible genotoxic effects of RF radiation have been extensively reviewed in several review articles and meta-analyses (see Lai, 2007; Vijayalaxmi and Prihoda, 2008, 2012, 2019; Ruediger, 2009; Verschaeve et al., 2010; IARC, 2013). Endpoints used to assess RF genotoxicity typically included DNA damage, DNA damage repair, micronucleus formation, chromosomal aberration, sister chromatids exchange, aneuploidy, and mutation rate. Overall, RF genotoxicity findings are inconsistent and sometimes contradicting. For example, while some studies reported statistically significant increases in genotoxicity, other studies reported no effects, and others reported statistically significant decreases. This inconsistency could be explained by the differences in the used test models, exposure parameters, and analytical methods (Ruediger, 2009; Verschaeve et al., 2010).

In their meta-analyses, Vijayalaxmi and Prihoda (2008, 2012, 2019) pointed out that the weighed genotoxicity outcome of RF exposure is very small. They indicated that the studies conducted within the generally recommended RF exposure guidelines mainly reported no effects. They also noticed that the statistically significant increases in some genotoxicity endpoints primarily occurred in experiments with small sample sizes and were largely influenced by publication bias. In their doctoral theses, Luukkonen (2011) and Herrala (2018) reviewed studies investigating possible genotoxic effects of RF radiation published between 2006 and 2018. They found that results were largely inconsistent and highlighted the possibility that many of the increased-genotoxicity findings may be due to possible thermal effects, primarily when high levels of SAR were used.

For experimental genotoxicity studies published in 2018 or later, the literature was reviewed according to the methodology described in appendix 1. Thirty-seven studies were published assaying genotoxic endpoints in response to RF radiation (Table 1). The majority of the studies (19 out of 37) were performed at *in vitro* level. Most *in vitro* studies used mammalian cells; however, one study (Barbora et al., 2021) used *Saccharomyces cerevisiae* yeast. Mammalian *in vivo* studies were performed using mice, rats, or rabbits. Non-mammalian *in vivo* studies used quail embryos (Yakymenko et al., 2018) and *Drosophila melanogaster* larvae (Yanagawa et al., 2020). *Allium cepa L*. onion was used by Chandel et al. (2019a,b) and Kumar et al. (2020).

Most of the studies examined exposure to RF radiation at the frequency range 900 MHz – 2.45 GHz. However, three studies used higher frequencies: 40 GHz (Koyama et al., 2019), 90 GHz (Barbora et al., 2021), and 150 GHz (Franchini et al., 2018). The modulation types used in the reviewed studies mainly included GSM, LTE, and UMTS. Experiments were performed at SAR levels between 0.004 and 10 W/kg. However, eight studies did not report SAR levels (Franchini et al., 2018; Yakymenko et al., 2018; Koyama et al., 2019; Panagopoulos 2019, 2020; Yanagawa et al., 2020; Barbora et al., 2021; Ioniță et al. 2021); in these studies, the exposure intensity was expressed in power density ($W/cm^2$).

Twenty-seven studies (out of 37) reported effects. Interestingly, 34 of these studies indicated that RF exposure increased genotoxicity or potentiated genotoxicity caused by other agents. Conversely, only three studies showed that RF radiation was protective against genotoxicity (Falone et al., 2018; Jin et al., 2021; Zeni et al., 2021). In these studies, RF exposure was found to decrease DNA damage induced by known genotoxic agents such as menadione, bleomycin, or ionizing radiation. The effects of RF exposure did not seem to be associated with the applied level of SAR. RF effects have been observed at both low levels (0.0054 W/kg; Pandey and Giri 2018) and high levels of SAR (10 W/kg; Smith-Roe et al. 2020). Studies that reported no effects have also used low (0.004 W/kg; Durdik et al. 2019) and high (5 W/kg; Jeong et al. 2018) SAR levels.

Overall, the results of the reviewed studies are contrasting, but it seems that recent studies indicate more likeliness of increased genotoxicity than those in earlier reviews (Verschaeve et al., 2010; Luukkonen, 2011; Herrala, 2018).

Table 1. Studies assessing genotoxicity of RF radiation published in 2018 or later.

| Reference | Assayed genotoxic endpoint(s) | RF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Al-Serori et al. 2018 | DNA damage and DNA damage repair in human U87, U251, U373 glioblastoma cells, SH-SY5Y neuroblastoma cells, NCH421k glioblastoma stem-like cells, ES-1 diploid fibroblast cells, TR-146 buccal cells, and HepG2 hepatocellular carcinoma cells | 1.95 GHz (UMTS), SAR 0.25, 0.5, or 1 W/kg for 16 h, intermittent (5 min on/10 min off) | None | When grown under serum-free conditions, RF exposure increased DNA damage in U87 glioblastoma cells. However, the induced DNA was transient and rapidly disappeared via the activation of nucleotide excision repair. |
| *Falone et al. 2018 | DNA damage in human SH-SY5Y neuroblastoma cells | 1.95 GHz (UMTS), SAR 0.3 or 1.25 W/kg for 20 h | Menadione (after RF exposure) | Sole exposure to RF radiation had no effect. However, RF exposure reduced menadione-induced DNA damage. |
| *Franchini et al. 2018 | DNA damage and aneuploidy in human dermal fibroblasts | 150 GHz (pulsed at 2.5 Hz), SAR 0.40 mW/cm$^2$ for 20 min | None | No effect was observed on DNA damage. However, RF exposure increased aneuploidy. |
| *Ding et al. 2018 | DNA strand breaks and oxidative DNA damage in human spermatozoa | 2.45 GHz (Wi-Fi), SAR 1 – 2.5 W/kg for 45 or 90 min | Trolox (during RF exposure) | Sole RF exposure increased DNA strand breaks and oxidative DNA damage. Trolox alleviated RF radiation-induced genotoxicity. |
| *Houston et al. 2018 | DNA strand breaks and oxidative DNA damage in mouse spermatogonial GC1, spermatocyte GC2 cells, and mature cauda mouse spermatozoa. | 1.8 GHz (CW), SAR 0.15 W/kg for 1, 2, 3, 4, 5, or 6 h | None | RF exposure had no effects on spermatogonial GC1 and spermatocyte GC2 cells. However, in mature cauda mouse spermatozoa, 3-h RF exposure increased DNA strand breaks, and 4-h RF exposure increased oxidative DNA damage. |
| †Jeong et al. 2018 | DNA damage in brain tissues from exposed female C57BL/6 mice | 1.95 GHz (WCDMA), WBA-SAR 5 W/kg for 2 h/d, 5 d/wk for 8 mo | None | No effect |
| *Li et al. 2018 | DNA damage in mouse spermatocyte GC2 cells | 1.8 GHz (GSM), SAR 1, 2, and 4 W/kg for 24 h, intermittent (5 min on/10 min off) | None | RF exposure at 4 W/kg increased DNA damage. |
| †Okatan et al. 2018 | Oxidative DNA damage in leukocytes and ovaries from exposed female Sprague-Dawley rats | 900 MHz (GSM), WBA-SAR 0.0096 – 0.0098 W/kg for 1 h/d for 25 d | None | RF exposure increased oxidative DNA damage in leukocytes. However, no effect was observed in the ovaries. |

Table 1. Continued

| Reference | Assayed genotoxic endpoint(s) | RF exposure | Co-exposure | Response |
|---|---|---|---|---|
| †Pandey and Giri 2018 | DNA damage in testes from exposed male Swiss albino mice | 900 MHz (GSM), WBA-SAR 0.0054 – 0.0516 W/kg for 3 h twice/d for 35 d | Melatonin (during RF exposure) | Sole exposure to RF radiation increased DNA damage. Melatonin diminished RF radiation-induced DNA damage. |
| *Su et al. 2018 | DNA damage in rat primary astrocytes, microglia, and cortical neurons | 1.8 GHz (GSM), SAR 4 W/kg for 24 h, intermittent (5 min on/10 min off) | None | No effect |
| †Yakymenko et al. 2018 | DNA strand breaks in embryonic cells and oxidative DNA damage in the tissues from 1-day old chicks after *in ovo* exposure of Japanese Quail embryos | 1.8 GHz (GSM), 0.32 μW/cm$^2$ for 19 d before and during the incubation period, intermittent (48 s on/ 12 s off) | None | RF exposure increased DNA strand breaks and oxidative DNA damage. |
| †Alkis et al. 2019a | DNA strand breaks and oxidative DNA damage in blood and brain tissues from exposed male Sprague-Dawley rats | 900 MHz (GSM), WBA-SAR 0.638 W/kg; 1.8 GHz (GSM), WBA-SAR 0.166 W/kg; or 2.1 GHz (GSM), WBA-SAR 0.174 W/kg; for 2 h/d for 6 mo | None | RF exposure increased DNA strand breaks and oxidative DNA damage. |
| †Alkis et al. 2019b | DNA strand breaks and oxidative DNA damage in testes from exposed male Sprague-Dawley rats | 900 MHz (GSM), WBA-SAR 0.638 W/kg; 1.8 GHz (GSM), WBA-SAR 0.166 W/kg; or 2.1 GHz (GSM), WBA-SAR 0.174 W/kg; for 2 h/d for 6 mo | None | RF exposure increased DNA strand breaks at 1.8 and 2.1 GHz while increased oxidative DNA damage was observed at all frequencies. |
| †Chandel et al. 2019a | DNA damage and CA in roots from exposed *Allium cepa L.* onion | 2.1 GHz (CW), SAR 0.282 W/kg for 1 or 4 h | None | Exposure to RF radiation for 4 h increased CA and DNA damage. |
| †Chandel et al. 2019b | DNA damage and CA in roots from exposed *Allium cepa L.* onion | 2.35 GHz (CW), SAR 0.313 W/kg for 1, 2, or 4 h | None | Exposure to RF radiation for 2 and 4 h increased CA and DNA damage. |
| *Durdik et al. 2019 | DNA damage in human umbilical cord blood cells | 900 MHz (GSM or UMTS), SAR 0.004 or 0.04 W/kg for 1 – 17 h | None | No effect |
| †Houston et al. 2019 | DNA strand breaks and oxidative DNA damage in spermatozoa from exposed male C57BL/6 mice. | 905 MHz (CW), WBA-SAR 2.2 W/kg for 12 h/d for 1, 3, or 5 wk | None | RF exposure increased DNA strand breaks and oxidative DNA damage. |

Table 1. Continued

| Reference | Assayed genotoxic endpoint(s) | RF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Jooyan et al. 2019 | DNA damage and MN in Chinese hamster ovary cells | 900 MHz (GSM), SAR 2 W/kg for 4, 12, or 24 h | None | No effects were observed on MN formation. However, exposure to RF radiation for 24 h increased DNA damage in cells directly exposed and bystander cells that received media from RF-exposed cells. |
| *Koyama et al. 2019 | DNA damage and MN in human corneal epithelial and human lens epithelial cells | 40 GHz, 1 mW/cm$^2$ for 24 h | None | No effect |
| *Panagopoulos 2019 | CA in human peripheral blood lymphocytes | 1.92 – 1.96 GHz (UMTS), 92 ± 27 μW/cm$^2$ for 15 min | None | RF exposure increased CA. |
| *Choi et al. 2020 | DNA damage in human adipose tissue-derived stem cells | 1.7 GHz (LTE), SAR 1 or 2 W/kg for 72 h | None | No effect |
| *Gulati et al. 2020 | DNA damage and mutations in *TP53* in human lymphocytes | 1.923, 1.947, and 1.977 GHz (UMTS), SAR 0.04 W/kg for 1 or 3 h | None | RF exposure resulted in a small increase in DNA damage in a frequency-dependent manner. The increase was maximal at 1.977 GHz. No mutations were observed in *TP53*. |
| †Kumar et al. 2020 | DNA damage and CA in roots from exposed *Allium cepa L.* onion | 900 MHz or 1.8 GHz (CW), SAR 0.0902 or 0.169 W/kg for 0.5 h, 1 h, 2 h, and 4 h | None | RF exposure increased DNA and CA. |
| †Lerchl et al. 2020 | DNA damage in brain, liver, and lung of fetuses of exposed female C57Bl/6 N mice (*in utero* exposure) | 1.960 GHz (UMTS), WBA-SAR 0.04 or 0.4 W/kg for 192, 204, or 240 h | Ethylnitrosourea | No effect |
| *Panagopoulos 2020 | CA in human peripheral blood lymphocytes | 1.92 – 1.96 GHz (UMTS), 92 ± 27 μW/cm$^2$ for 15 min | Caffeine (during RF exposure) | RF exposure increased CA and potentiated caffeine-induced CA. |
| *Regalbuto et al. 2020 | DNA damage in human fibroblasts | 2.45 GHz (CW or PW), SAR 0.7 W/kg for 2 h | None | No effect |
| †Sharma et al. 2020 | DNA damage in brian tissues from exposed male Wistar rats | 1.8 GHz, SAR 0.433 W/kg for 4 h/d for 90 d | None | RF exposure increased DNA damage. |
| †Sharma and Shukla 2020 | DNA damage in brian tissues from exposed male Wistar rats | 900 MHz, SAR 0.231 W/kg for 1 h, 2 h, or 4 h/d for 90 d | None | RF exposure increased DNA damage. |

Table 1. Continued

| Reference | Assayed genotoxic endpoint(s) | RF exposure | Co-exposure | Response |
|---|---|---|---|---|
| †Smith-Roe et al. 2020 | DNA damage in brain and liver tissues and peripheral blood leukocytes, and MN in peripheral blood erythrocytes from exposed Sprague-Dawley rats and B6C3F1/N mice | 900 MHz (mice) or 1.9 (rats) (CDMA or GSM), WBA-SAR 1.5, 2.5, 3, 5, 6 or 10 W/kg, for 18 h/d for 19 (rats) or 14 (mice) wk, intermittent (10 min on/10 min off) | None | RF exposure increased DNA damage in tissues from different regions in mice brains. An equivocal increase in DNA damage was observed in other tissues of mice and rats. No effect on MN was observed. |
| †Yang et al. 2020 | DNA damage in Cochlear Stria marginal cells from exposed Sprague-Dawley rats | 1.8 GHz (GSM), WBA-SAR 2 or 4 W/kg for 24 h, intermittent (5 min on/ 10 min off) | None | No effect |
| †Yanagawa et al. 2020 | The mutation rate in *Drosophila melanogaster* larvae | 2.45 GHz, 288.8 ± 1.11 μW/cm$^2$ or 78.8 ± 14.64 mW/cm$^2$ for 3 min | None | RF exposure increased the mutation rate. |
| †Zhu et al. 2020 | DNA damage in the brain, heart, liver, lung, kidney, and stomach from exposed rabbits | 1.8 GHz (GSM), WBA-SAR 0.7 W/kg for 6 h/d, 6 d/wk for 16 wk | None | RF exposure increased DNA damage only in brain tissues. |
| †Alkis et al. 2021 | DNA strand breaks and oxidative DNA damage in the liver from exposed male Sprague-Dawley rats | 1.8 GHz (GSM), WBA-SAR 0.62 W/kg; 2.1 GHz (GSM), WBA-SAR 0.2 W/kg; for 2 h/d for 7 mo | None | RF exposure increased DNA strand breaks and oxidative DNA damage. |
| *Barbora et al. 2021 | DNA damage in *Saccharomyces cerevisiae* yeast | 90 GHz, 1 mW/cm$^2$ for 6 h | None | No effect |
| *Ioniţă et al. 2021 | DNA damage in mouse pre-B lymphocytes | 950 MHz, 0.07 or 0.28 mW/cm$^2$ for 48 h | None | No effect |
| *Jin et al. 2021 | DNA damage in mouse B16 melanoma cells, human immortalized HaCaT keratinocytes, and human MNT-1 melanoma cells | 1.762 GHz (LTE), SAR 8 W/kg for 3 or 24 h. | Bleomycin or ionizing radiation (before RF exposure) | Sole exposure to RF radiation had no effect. However, RF exposure decreased bleomycin- and ionizing radiation-induced damage. |

Table 1. Continued

| Reference | Assayed genotoxic endpoint(s) | RF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Zeni et al. 2021 | DNA damage in human SH-SY5Y neuroblastoma cells (cells were directly exposed or bystander exposed by culturing in the medium of the exposed cells) | 1.95 GHz (UMTS), SAR 0.3 W/kg for 20 h | Menadione (after RF exposure) | Sole exposure to RF radiation had no effect. However, RF exposure decreased menadione-induced DNA damage in both directly exposed and bystander cells. |

Asterisks* indicate *in vitro* studies, and daggers † indicate *in vivo* studies.

Centimeter (cm), Chromosomal aberration (CA), Code Division Multiple Access (CDMA), Continuous-wave (CW), Day (d), Deoxyribonucleic acid (DNA), Gigahertz (GHz), Global System for Mobile Communications (GSM), Hour (h), Kilogram (kg), Long-Term Evolution (LTE), Megahertz (MHz), Minute (min), Micronuclei (MN), Month (mo), Pulsed-wave (PW), Radiofrequency (RF), Second (s), Specific absorption rate (SAR), Universal Mobile Telecommunications Service (UMTS), Watt (W), Whole-body averaged specific absorption rate (WBA-SAR), Wireless Fidelity (Wi-Fi), Wideband Code Division Multiple Access (WCDMA), Week (wk).

## 1.7 SUMMARY OF GENOTOXICITY FINDINGS LINKED TO ELF MFs

Studies that investigated possible genotoxic effects of ELF MFs have been extensively reviewed by, e.g., IARC (2002), Juutilainen et al. (2006), Lai (2007), World Health Organization (WHO, 2007), Vijayalaxmi and Prihoda (2009), Luukkonen (2011) and Herrala (2018). In the reviewed studies, the assayed genotoxic endpoints mainly included DNA damage, DNA damage repair, micronucleus formation, aneuploidy, chromosomal aberration, sister chromatid exchange, and microsatellite mutations.

IARC (2002) and WHO (2007) indicated the lack of evidence for a direct genotoxic effect from sole exposure to ELF MFs, except for extremely strong fields. However, in their meta-analysis, Juutilainen et al. (2006) reviewed experimental studies where MF exposure was combined with known genotoxic agents and found that most of the studies reported MF effects in such a combined setting. Interestingly, the reported effects followed a non-linear dose-response, showing a minimum percentage of findings at fields between 1 and 3 mT. Therefore, they postulated that the RPM could be an explanation for such a biphasic dose-response relationship.

In their doctoral theses, Luukkonen (2011) and Herrala (2018) reviewed the studies investigating possible genotoxic effects of MFs published between 2006 and 2018. They concluded that co-exposure increases the likelihood of finding MF effects, particularly in fields with an intensity of $\leq$ 1 mT. In addition, it was indicated that there is an increased likelihood of observing MF effects in stronger fields or after prolonged exposure durations (longer than a week in animal studies; Herrala, 2018).

For experimental genotoxicity studies published in 2018 or later, the literature was reviewed according to the methodology described in appendix 1. Twelve studies were published assaying genotoxic endpoints in response to ELF MFs (Table 2). Most of these studies were performed using mammalian cells at *in vitro* level. However, in their experiments, Heredia-Rojas et al. (2018) used male BALB/c mice. Wang et al. (2019) used Sprague-Dawley rats (*in vivo*) in addition to human AC16 cardiomyocytes (*in vitro*). Stankevičiūtė et al. (2019) used different species of aquatic animals. *Saccharomyces cerevisiae* yeast was used in three studies (Mercado-Sáenz et al., 2019, 2021; Burgos-Molina et al., 2020). Most of the studies used a frequency of 50 Hz. Other frequencies were 60 Hz (Heredia-Rojas et al., 2018; Song et al., 2018), 5 Hz (Ross et al., 2018), and 25 Hz (Mercado-Sáenz et al., 2019, 2021). Experiments were performed at flux density between 0.005 and 6 mT. All of the studies were performed without co-exposures. Four studies reported that MFs increased genotoxicity (Heredia-Rojas et al., 2018; Stankevičiūtė et al., 2019; Samiei et al., 2020; Mercado-Sáenz et al. 2021), whereas two studies reported that MF exposure decreased spontaneous mutations and enhanced the DNA repair activity in *Saccharomyces cerevisiae* yeast. The other six studies indicated that MFs had no effect (Ross et al., 2018; Song et al., 2018; Sun et al., 2018; Verschaeve et al., 2019; Wang et al., 2019; Lv et al., 2021). In conclusion, the results of the reviewed studies are discrepant, and no consistent evidence was found for ELF MF genotoxic effects.

Table 2. Studies assessing genotoxicity of ELF MFs published in 2018 or later.

| Reference | Assayed genotoxic endpoint(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| †Heredia-Rojas et al. 2018 | MN in male BALB/c mice | 60 Hz, 0.0088 or 2 mT for 3 or 10 d | None | MF exposure increased MN formation. Exposure to 2-mT MFs for 3 d produced the highest response. |
| *Ross et al. 2018 | Chromosomal breaks in human mesenchymal stromal cells | 5 Hz, 0.4 mT for20 min/day, 3 d/wk for 2 wk | None | No effect |
| *Song et al. 2018 | DNA damage in human cervical cancer HeLa cells and lung fibroblast IMR-90 cells | 60 Hz, 3 or 6 mT for 30 min or 1 h (single exposure), or 30 min/d for 3 d (repetitive exposure). | None | No effect |
| *Sun et al. 2018 | DNA damage in mouse embryonic fibroblasts proficient or deficient in ataxia-telangiectasia mutation | 50 Hz, 2 mT for 15 min, 1 h, or 24 h | None | No effect |
| *Mercado-Sáenz et al. 2019 | Spontaneous mutation in *Saccharomyces cerevisiae* yeast | 50 Hz (sinusoidal), 2.45 mT continuously for 40 d or 25 Hz (pulsed), 1.5 mT for 8/d for 40 d | None | Exposure to 25 Hz pulsed MFs decreased spontaneous mutation in *Saccharomyces cerevisiae* yeast. 50 Hz MF exposure had no effect. |
| †Stankevičiūtė et al. 2019 | MN, NB, NBF, and BL in *Oncorhynchus mykiss* rainbow trout, *Hediste diversicolor* ragworm, and *Limecola balthica* Baltic clam | 50 Hz, 1 mT for 12 or 40 d | None | MF exposure increased the assayed genotoxic parameters in all test species. The strongest response was in *Limecola balthica* Baltic clam |
| *Verschaeve et al. 2019 | MN in human hepatoma-derived C3A cells | 50 Hz, 0.005, 0.01, 0.05, 0.1, or 0.5 mT for 24 h | None | No effect |
| ‡Wang et al. 2019 | DNA damage in human AC16 cardiomyocytes (*in vitro*) or cardiac tissues from exposed male Sprague-Dawley rats (*in vivo*) | 50 Hz, 0.1 mT. *In vitro*: continuous exposure for 1 h or intermittent exposure for 1.25 h (15 min on/15 min off). *In vivo*: continuous exposure for 7 d | None | No effect |
| *Burgos-Molina et al. 2020 | DNA repair activity in *Saccharomyces cerevisiae* yeast | 50 Hz, 2.45 mT for 21 d | None | MF exposure increased DNA repair activity. |
| *Samiei et al. 2020 | MN in human dental pulp stem cells | 50 Hz, 0.5 or 1 mT for 20 or 40 min/d for 7 d | None | MF exposure increased MN formation. |

Table 2. Continued

| Reference | Assayed genotoxic endpoint(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Lv et al. 2021 | DNA damage in human amniotic epithelial FL cells, skin fibroblast cells, and umbilical vein endothelial cells | 50 Hz, 0.4, 1, or 2 mT for 15 min, 1 h, or 24 h | None | No effect |
| *Mercado-Sáenz et al. 2021 | DNA damage and spontaneous mutation in *Saccharomyces cerevisiae* yeast | 25 Hz, 1.5 mT for 8 h/d for 40 d | None | MF exposure increased DNA damage and spontaneous mutation in *Saccharomyces cerevisiae* yeast. |

Asterisks* indicate *in vitro* studies, daggers † indicate *in vivo* studies, and double daggers ‡ indicate studies that involved both *in vitro* and in vivo experiments.
Cells with blebbed nuclei (BL), Day (d), Deoxyribonucleic acid (DNA), Hertz (Hz), Hour (h), Magnetic fields (MF), Micronuclei (MN), Millitesla (mT), Minute (min), Nuclear buds (NB), Nuclear buds on filament cells (NBF), Week (wk).

### 1.7.1 Effects of ELF MFs on gene transcription related to DNA damage signaling

This section pays particular attention to the previous studies investigating the effect of MFs on the transcription of genes involved in DNA damage signaling. For this purpose, the literature has been reviewed according to the methodology described in Appendix 2. Twenty-nine published studies (Table 3) explored the transcription pattern of 19 genes (Fig. 2). Most of these studies were performed using mammalian cells at *in vitro* level. However, there were also *in vivo* studies, such as Li et al., 2014 (*Danio rerio* Zebrafish), Urnukhsaikhan et al. 2017 (C57B6 mice), and Wang et al., 2019 (Sprague-Dawley rats). In addition, Lin et al. (2016) used *Saccharomyces cerevisiae* yeast as a non-mammalian *in vitro* model.

Most of the studies used the frequency of 50 Hz (22 out of 29). The other frequencies used were 60 Hz (Loberg et al., 1991; Huang et al., 2014a,b; Lee et al., 2015; Urnukhsaikhan et al., 2016, 2017) and 10 Hz (Ashta et al., 2020). Experiments were performed at flux densities between 0.0012 and 10 mT. Combining MFs with other agents was used in 11 out of the 29 studies, whereas 18 studies were conducted without co-exposure. Co-exposures mainly included DNA-damaging chemicals such as cisplatin, bleomycin, and temozolomide.

Sixteen studies (out of the 29) reported effects on DNA damage signaling-related gene transcription (either up- or down-regulation), while 13 studies did not report any effects. Interestingly, all studies that used co-exposures (except Cheng et al., 2017) reported one or more events of altered gene transcription. However, notably, all studies that did not report any effect (except Cheng et al., 2017) were performed without co-exposures. Increasing the intensity of the applied MFs did not seem to be associated with an increased likelihood of effects on gene transcription. In fact, the majority of the studies that reported no effects (7 out of 13) used a flux density > 1 mT, and the majority of the effect-reporting studies (9 out of 16) were performed at a flux density ≤ 1 mT.

Overall, the results of the reviewed studies are contrasting, and drawing a definite conclusion is not fully supported. However, there seems to be a tendency that combining ELF MFs with DNA-damaging chemicals increases the likelihood of finding MF effects on the transcription of DNA damage signaling genes, especially at intensities ≤ 1 mT. This is, to some extent, in line with previous reviews (IARC, 2002; Juutilainen et al., 2006; WHO, 2007; Luukkonen, 2011; Herrala, 2018).

Table 3. Published studies on the effects of ELF MFs on the transcription of DNA damage signaling-related genes.

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Loberg et al. 1991 | *BAX*, *CDKN1A*, *GADD45A*, and *TP53* in human normal mammary epithelial cells and HBL-100 and breast cancer cells | 60 Hz, 0.01, 0.1, or 1 mT for 20 min, 1, 4, or 24 h | None | No effect |
| *Czyz et al. 2004 | *Bcl2* and *Cdkn1a* in mouse embryonic stem cells | 50 Hz, 0.1, 1, or 2.3 mT for 6 or 48 h, intermittent (5 min on/10 min off or 5 min on/30 min off) | None | No effect |
| *Nikolova et al. 2005 | *Bax*, *Bcl2*, *Gadd45a*, and *Tp53* in mouse embryonic stem cells | 50 Hz, 2 mT for 48 h, intermittent (5 min on/30 min off) | None | Upregulation of *Bax* and *Bcl2* 5 days after MF exposure. Downregulation of *Gadd45a* 17 days after MF exposure. *Tp53* was not affected |
| *Girgert et al. 2009 | *BRCA1*, *CDKN1A*, and *TP53* in human MCF-7 breast cancer cells and MCF-7 cells transfected with the MT1 gene. | 50 Hz, 0.0012 mT for 48 h | Estradiol or melatonin (during MF exposure) | MF exposure downregulated *BRCA1*, *TP53* in estradiol-treated MCF-7 cells and *CDKN1A* in estradiol-treated MCF-7 transfected cells. Conversely, MF exposure upregulated *TP53* in MCF-7 transfected cells simultaneously treated with estradiol and melatonin. |
| *Marcantonio et al. 2010 | *CDKN1A* in human BE(2)C neuroblastoma cells | 50 Hz, 1 mT for 6 or 24 h | ATRA (during MF exposure) | MF exposure enhanced ATRA-induced upregulation of *CDKN1A*. |
| *Sun et al. 2010 | *BAX*, *BCL2*, and *TP53* in human first-trimester villous trophoblasts | 50 Hz, 0.4 mT for 6, 48, or 72 h | None | No effect |
| *Huang et al. 2014a | *CDKN1A* and *CHEK2* in human immortalized HaCaT keratinocytes transfected with CHK2 siRNA or scrambled siRNA | 60 Hz, 1.5 mT for 96, 120, or 144 h | None | MF exposure Upregulated *CDKN1A* in cells transfected with *CHK2* siRNA and Downregulated *CHEK2* in cells transfected with scrambled siRNA. |

Table 3. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Huang et al. 2014b | *CDC20*, *CDC25B*, *CDKN1A*, and *GADD45A* in human primary normal epidermal keratinocytes | 60 Hz, 1.5 mT for 96, 120, or 144 h | None | Not effect |
| †Li et al. 2014 | *tp53* in embryos of *Danio rerio* Zebrafish | 50 Hz, 0.03, 0.1, 0.2, 0.4, 0.8 mT for 96 h | None | No effect |
| *Ma et al. 2014 | *Cdkn1a*, *Gadd45a*, and *Tp53* in mouse embryonic neural stem cells | 50 Hz, 2 mT for 3 d, intermittent (5 min on/10 min off) | None | No effect |
| *Lee et al. 2015 | *TP53INPI* in human MCF-7 breast cancer cells | 60 Hz, 2 mT for 16 h | None | No effect |
| *Baharara et al. 2016 | *TP53* in human A2780 ovarian adenocarcinoma cells | 50 Hz 0.1 mT for 2 h | Cisplatin (during MF exposure) | MF exposure upregulated *TP53* in cisplatin-treated cells. |
| *Lin et al. 2016 | *DDR48* and *RTT107* in *Saccharomyces cerevisiae* yeast | 50 Hz, 6 mT for 96 h | None | No effect |
| *Ma et al. 2016 | *Bax* and *Bcl2* in mouse embryonic neural stem cells | 50 Hz, 1 mT for 3 d | None | No effect |
| *Sanie-Jahromi et al. 2016 | *GADD45A*, *LIG4*, *PRKDC*, *XRCC1*, *XRCC4*, *XRCC5*, and *XRCC6* in human MCF-7 breast cancer cells and SH-SY5Y neuroblastoma cells | 50 Hz, 0.25, 0.5 mT for 30 min, continuously or intermittently (5 min on/5 min off or 15 min on/15 min off) | Cisplatin (during MF exposure) | MF exposure tended to downregulate the assayed genes in the absence or the presence of cisplatin. The magnitude of the effect differed according to cell type, flux density, assay time point, and waveform. |
| *Urnukhsaikhan et al. 2016 | *BAX* in human bone marrow mesenchymal stem cells | 60 Hz, 10 mT for 3 h | None | MF exposure upregulated *BAX*. |
| Cheng et al. 2017 | *TP53* in human umbilical vein endothelial cells | 50 Hz, 2.25 mT for 15 min | Hydroxytyrosol (during MF exposure) | No effect |

Table 3. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| Jeong et al. 2017 | *SIRT1* in in human bone marrow mesenchymal stem cells | 50 Hz, 1 mT for 12 days | None | MF exposure upregulated *SIRT1*. |
| *Sanie-Jahromi and Saadat 2017 | *GADD45A*, *LIG4*, *PRKDC*, *XRCC1*, *XRCC4*, *XRCC5*, and *XRCC6* in human MCF-7 cancer cells and SH-SY5Y neuroblastoma cells | 50 Hz, 0.5 mT for 30 min, intermittent (15 min on/15 min off) | Bleomycin and cisplatin (during MF exposure) | MF exposure resulted in sporadic dysregulations of the assayed genes in the chemically treated cells. |
| *Sanie-Jahromi et al. 2017 | *LIG4*, *PRKDC*, *XRCC4*, *XRCC5*, and *XRCC6* in human SH-SY5Y neuroblastoma cells | 50 Hz, 0.5 mT for 30 min, intermittent (15 min on/15 min off) | β-Lapachone and morphine (during MF exposure) | MF exposure did not affect β-Lapachone-induced dysregulation. However, MF exposure tended to upregulate the assayed genes in cells simultaneously co-treated with β-Lapachone and morphine. |
| †Urnukhsaikhan et al. 2017 | *Bax* in C57B6 mice | 60 Hz, 10 mT for 3 h/d for 3 d, pulsed | Ischemic stroke (before MF exposure) | MF exposure downregulated *Bax* in stroke ischemic-mice |
| *Consales et al. 2018 | *TP53* in human SH-SY5Y neuroblastoma cells and mouse primary cortical neurons | 50 Hz, 1 mT for 4, 6, 16, 24, 48, or 72 h | None | No effect |
| *Han et al. 2018 | *CDKN1A*, *BAX*, *BCL2*, and *TP53* in human MCF-7 breast cancer cells and MCF-10A normal breast epithelial cells | 50 Hz, 1 mT for 12 h | None | No effect |
| *Sanie-Jahromi and Saadat 2018 | *GADD45A*, *LIG4*, *PRKDC*, *XRCC1*, *XRCC4*, *XRCC5*, and *XRCC6* in human MCF-7 cancer cells and SH-SY5Y neuroblastoma cells | 50 Hz, 0.5 mT for 30 min, intermittent (15 min on/15 min off) | cisplatin and morphine (during MF exposure) | MF exposure did not affect morphine-induced dysregulation. However, MF exposure downregulated *LIG4*, *PRKDC*, *XRCC4*, *XRCC5*, *XRCC6* in cells simultaneously co-treated with morphine and cisplatin. |

Table 3. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| ‡Wang et al. 2019 | *TP53* in human AC16 cardiomyocytes (*in vitro*), or cardiac tissues from exposed male Sprague-Dawley rats | 50 Hz, 0.1 mT. *In vitro*: continuously for 1 h or intermittently for 1.25 h (15 min on/15 min off). *In vivo*: continuously for 7 d | None | No effect |
| *Ashta et al. 2020 | *TP53* in human A172 glioblastoma cells | 10 Hz, 5 mT, for 96 or 120 h, square waves | Temozolomide (during MF exposure) | Sole MF exposure upregulated *TP53*. In addition, MF exposure enhanced temozolomide-induced upregulation of *TP53*. |
| *Consales et al. 2021 | *CDKN1A* in differentiating human SH-SY5Y neuroblastoma cells cultured in 3D or traditional 2D cultures | 50 Hz, 1 mT for 72 h | None | MF exposure upregulated *CDKN1A* only in 3D cultures. |
| *Dehghani-Soltani et al. 2021 | *TP53* in human T98 and A172 glioblastoma cells | 50 Hz, 7 mT for 6 h/d for 2 or 3 d | Temozolomide (during MF exposure) | MF exposure enhanced temozolomide-induced upregulation of *TP53*. |
| *Heidari et al. 2021 | *BCL2* in human gastric adenocarcinoma cells | 50 Hz, 0.2 or 2 mT for 18 h, continuously or Intermittently (1.5 h on/1.5 h off) | None | MF exposure downregulated *BCL2*. |

Asterisks* indicate *in vitro* studies, daggers † indicate *in vivo* studies, and double daggers ‡ indicate studies that involved both *in vitro* and *in vivo* experiments.
All-trans retinoic acid (ATRA), Day (d), Hertz (Hz), Hour (h), Magnetic fields (MFs), Millitesla (mT), Minute (min).
Assayed DNA damage signaling-related genes: B-cell lymphoma 2 (*BCL2*), BCL2-associated X protein (*BAX*), Breast cancer gene (*BRCA*), Cell division cycle 20 (*CDC20*), Cell division cycle 25B (*CDC25B*), Checkpoint Kinase 2 (*CHEK2*), Cyclin-dependent kinase inhibitor 1A (*CDKN1A*), DNA damage-responsive protein 48 (DDR48), DNA ligase 4 (*LIG4*), Growth arrest and DNA-damage-inducible, alpha (*GADD45A*), Protein kinase, DNA-activated, catalytic polypeptide (*PRKDC*), Regulator of Ty1 transposition protein 107 (*RTT107*), Sirtuin 1 (*SIRT1*), Tumor protein p53 - induced nuclear protein 1 (*TP53INP1*), Tumor protein p53 (*TP53*), X-Ray Repair Cross Complementing 1 (*XRCC1*), X-Ray Repair Cross Complementing 4 (*XRCC4*), X-Ray Repair Cross Complementing 5 (*XRCC5*), ), X-Ray Repair Cross Complementing 6 (*XRCC6*).

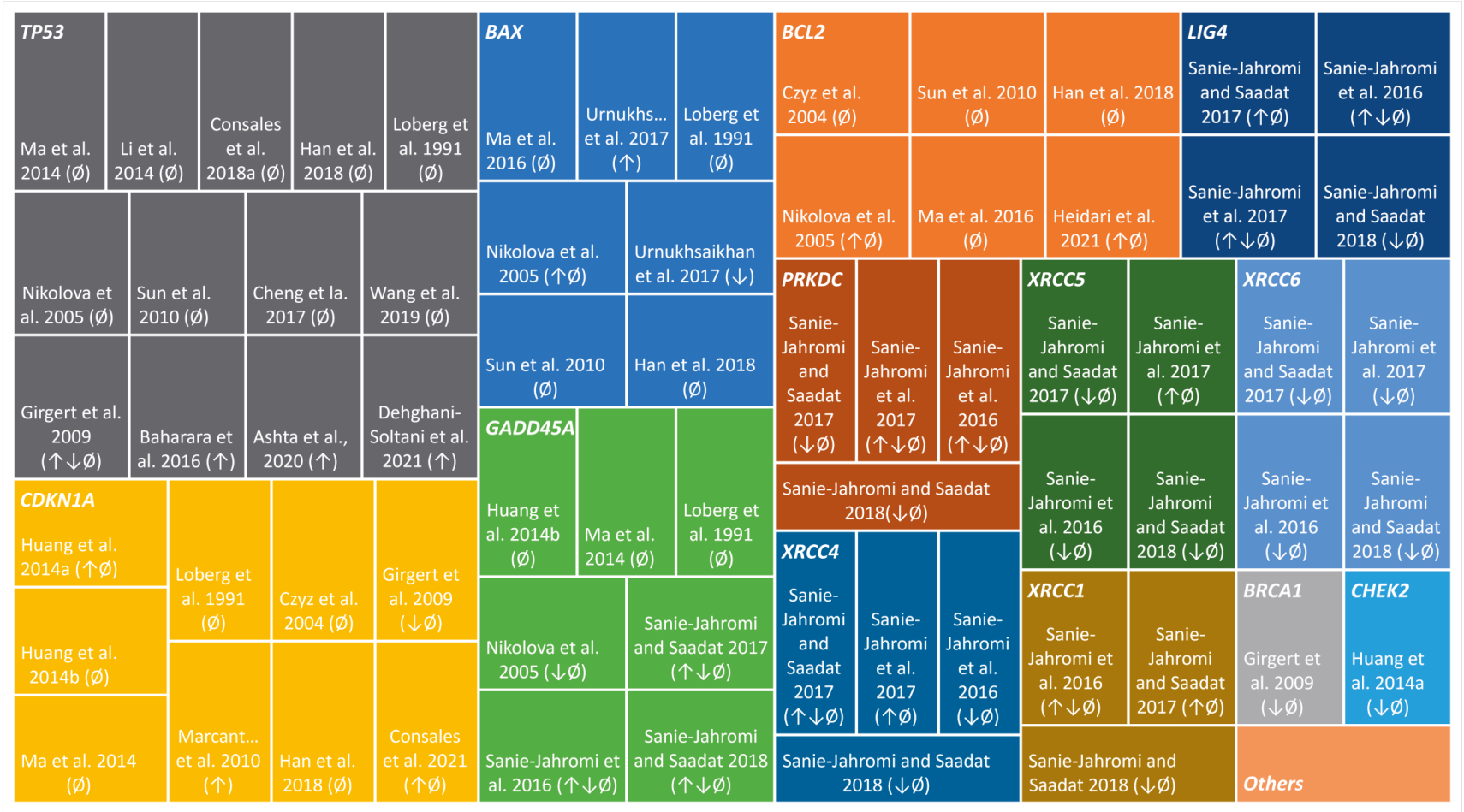


Figure 2. Treemap summarizing studies on ELF MFs effects on the transcription of DNA damage signaling-related genes. The arrows indicate the direction of the dysregulation (↑: upregulation, ↓: downregulation), whereas the symbol Φ indicates no effect. (Online version in color).

## 1.8 SUMMARY OF OXIDATIVE STRESS FINDINGS LINKED TO ELF MFs

Studies that explored the effects of ELF MFs on oxidative stress-related endpoints have been extensively reviewed by, e.g., WHO (2007), Luukkonen (2011), Wang and Zhang (2017), and most recently, Schuermann and Mevissen (2021). In the reviewed studies, the assayed oxidative stress-related endpoints typically included free radical production, lipid peroxidation, total antioxidant status, levels of glutathione and oxidized glutathione, and the activity of oxidative stress-related enzymes (e.g., catalase, superoxide dismutase, peroxidase, or glutathione reductase). ELF MFs have been reported to increase oxidative stress in most of the investigations. This increase has been shown in mammalian cells, tissues of rats and mice exposed to MFs, and various non-mammalian test systems. However, it should be noted that fewer investigations indicated that MFs reduced or had no effect on oxidative stress (Wang and Zhang, 2017; Schuermann and Mevissen, 2021).

The present section focuses on the studies investigating the effect of ELF MFs on the transcription of genes involved in oxidative stress, i.e., genes involved in the formation and metabolism of free radicals and defense against oxidative stress. For this purpose, the literature has been reviewed according to the methodology described in Appendix 2. Forty-nine published studies (Table 4) explored the transcription pattern of 48 genes (Fig. 3). Most of these studies were performed *in vitro* using mammalian cells (33 out of 49 studies). Two *in vitro* studies used budding yeast and *Irpex lacteus* fungi as non-mammalian models (Lian et al., 2018; Sun et al., 2019; respectively). Mammalian *in vivo* studies were in Sprague-Dawley rats (Falone et al., 2008; George et al., 2008; Zhuo et al., 2019; Wang et al., 2019), Wistar rats (Saadat et al., 2010), CD1 mice (Mariucci et al., 2010; Villarini et al., 2013), and amyotrophic lateral sclerosis mouse models (Liebl et al., 2015). Non-mammalian *in vivo* studies used *Drosophila* larvae and flies (Tipping et al., 1999; Li et al., 2013; Zhang et al., 2016), *Caenorhabditis elegans* worms (Miyakawa et al., 2001; Wang et al., 2020), and *galloprovincialis* mussels (Malagoli et al., 2006).

50 Hz was the predominantly investigated frequency. It was used in 38 out of the 49 studies. The other investigated frequencies were 60 Hz (Lin et al., 1998; Balcer-Kubiczek et al., 2000; Miyakawa et al., 2001; George et al., 2008; Lee et al., 2015; Kim et al., 2017), 75 Hz (De Mattei et al., 2009), 100 Hz (Akbarnejad et al., 2017), 16 Hz (Ehnert et al., 2017), 5 Hz (Vinhas et al., 2020) and 16.7 Hz (Groiss et al. 2021). Most of the studies were performed at a flux density ≤ 1 mT. However, 12 studies used a flux density > 1 mT (Balcer-Kubiczek et al., 2000; Miyakawa et al., 2001; De Mattei et al., 2009; Li et al., 2013; Lee et al., 2015; Zhang et al., 2016; Akbarnejad et al., 2017; Lian et al., 2018; Sun et al., 2019; Zhou et al., 2019; Wang et al., 2020; Vinhas et al., 2020), and three studies were performed with flux densities below and above 1 mT (Czyz et al., 2004; Villarini et al., 2013; Groiss et al., 2021). Twenty studies (out of 49) combined MFs with other factors, while 29 studies were performed without any co-exposure. Co-exposures were, in most cases, factors known to induce oxidative stress, such as aluminum chloride, thermal stress, or lipopolysaccharide.

Most of the studies (36 out of 49) found that MFs change the transcription of oxidative stress-involved genes, whereas 13 studies reported no effects. Unlike DNA damage signaling-related genes, combining MFs with other agents did not seem to be a prerequisite for observing an effect on oxidative stress-related genes. Indeed, the majority of the effect-reporting studies (20 out of 36) did not combine MFs with any other factor. Interestingly, upregulation was the more frequent direction of gene transcription alteration. There were 21 studies where only upregulation was reported (Lin et al., 1998; Miyakawa et al., 2001; Tokalov et al., 2003; Tokalov and Gutzeit 2004; Alfieri et al., 2006; Falone et al., 2007; Bernardini et al., 2007; Gottwald et al., 2007; George et al., 2008; Villarini et al., 2013; Wang et al., 2014; Fan et al., 2015; Zhang et al., 2016; Ehnert et al., 2017; Kim et al., 2017; Akbarnejad et al., 2017; Costantini et al., 2019; Sun et al., 2019; Zhou et al., 2019; Wang et al., 2020; Groiss et al., 2021).On the other hand, eight studies reported sole downregulation (Tipping et al., 1999; Reale et al., 2006; Falone et al., 2008; Vianale et al.,

2008; De Mattei et al., 2009; Aikins et al., 2017; Vinhas et al., 2020; Consales et al., 2021), and seven studies reported both up-and-down-regulations (Patruno et al., 2012; Li et al., 2013; Mahmoudinasab and Saadat 2016; Mahmoudinasab et al., 2016; Mahmoudinasab and Saadat 2018a; Mahmoudinasab and Saadat 2018b; Patruno et al., 2020). Of notice is that most of the affected genes are found to be antioxidant genes whose upregulation is involved in defense against increased oxidative stress, suggesting exposure to MFs increases oxidative stress.

Overall, because of the inconsistent findings (upregulations, downregulations, and no effects), drawing a definite conclusion is not totally justified; however, the results of the reviewed studies point to the possibility of increased oxidative stress by exposure to ELF MFs, and thus, to some extent, these results are in line with conclusions of previous reviews (Wang and Zhang, 2017; Schuermann and Mevissen, 2021).

Table 4. Published studies on the effects of ELF MFs on the transcription of oxidative stress-related genes.

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Lin et al. 1998 | *HSP70* in human HL60 promyelocytic leukemia cells | 60 Hz, 0.008 mT for 20 min | None | MF exposure upregulated *HSP70*. |
| †Tipping et al. 1999 | *Hsp70* in *Drosophila* larvae | 50 Hz, 0.008 mT for 20 min | None | MF exposure downregulated *Hsp70*. |
| *Balcer-Kubiczek et al. 2000 | *HSP70* and *SOD1* in human HL60 promyelocytic leukemia cells | 60 Hz, 2 mT for 3 or 24 h, sine- or square-wave | None | No effect |
| †Miyakawa et al. 2001 | *hsp-16* in *Caenorhabditis elegans* worms | 60 Hz, 0.5 T for 15, 30, 60, or 120 min | None | MF exposure upregulated *hsp-16*. |
| *Henderson et al. 2003 | *HSP60* in human umbilical vein endothelial cells | 50 Hz, 0.7 mT for 6 or 24 h | Thermal stress (before or after MF exposure) | No effect |
| *Tokalov et al. 2003 | *HSP27, HSP60, HSP70, HSP75, HSP78,* and *HSP90* in human HL60 promyelocytic leukemia cells | 50 Hz, 0.06 mT for 30 min | Thermal stress (during MF exposure) | Sole MF exposure upregulated *HSP27*, *HSP70*, *HSP75*, *HSP78*. *HSP70* showed the strongest response. In addition, MF exposure increased thermal stress-induced upregulation of these genes. No effect was observed on *HSP60* or *HSP90*. |
| *Coulton et al. 2004 | *HSP27*, *HSP70A*, and *HSP70B* in human leukocytes | 50 Hz, 0.02, 0.04, 0.06, 0.08, or 0.1 mT for 2 or 4 h | Heat (during MF exposure) | No effect |
| *Czyz et al. 2004 | *Hsp70* in mouse embryonic stem cells | 50 Hz, 0.1, 1, or 2.3 mT for 6 or 48 h, intermittent (5 min on/10 min off or 5 min on/ 30 min off) | None | No effect |
| *Tokalov and Gutzeit 2004 | *HSP27*, *HSP60*, *HSP70A*, *HSP70B*, *HSP70C*, *HSP75*, *HSP78*, *HSP90*$\alpha$, and *HSP90*$\beta$ in human HL60 promyelocytic leukemia cells | 50 Hz, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, or 0.14 mT for 30 min | Heat (during MF exposure) | Sole MF exposure upregulated *HSP70A*, *HSP70B*, and *HSP70C*. The applied thermal stress potentiated this effect. The induction was maximum at 0.06 – 0.08 mT and returned to the control level at 0.1 mT and 0.14 mT. No effect was observrd on *HSP27*, *HSP60*, *HSP75*, *HSP78*, *HSP90*$\alpha$, and *HSP90*$\beta$. |

Table 4. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Alfieri et al. 2006 | *HSP70* in pig pulmonary arteries endothelial cells | 50 Hz, 0.68 mT for 24 h | Thermal stress (after MF exposure) | MF exposure increased thermal stress-induced upregulation of *HSP70*. |
| †Malagoli et al. 2006 | *hsp70* in *Mytilus galloprovincialis* mussels | 50 Hz, 0.4 mT for 30 min | None | No effect |
| *Reale et al. 2006 | *NOS2* in human monocytes | 50 Hz, 1 mT overnight | LPS (during MF exposure) | MF exposure downregulated *NOS2* in LPS-stimulated cells. |
| *Bernardini et al. 2007 | *HSP27, HSP70,* and *HSP90* in pig aortic endothelial cells | 50 Hz, 1 mT for 4 h | None | MF exposure upregulated *HSP27, HSP70,* and *HSP90*. However, statistical significance was seen only for *HSP70*. |
| *Falone et al. 2007 | *CAT, GPX1*, *GSTP1*, and *SOD1* in human SH-SY5Y neuroblastoma cells | 50 Hz, 1 mT for 96 h | None | MF exposure upregulated *GPX1*. No effect was observed on *CAT*, *GSTP1*, or *SOD1*. |
| *Gottwald et al. 2007 | *HSP72* in human HL60 promyelocytic leukemia cells, rat H9c2 heart myoblast cells, and human Girardi heart cells | 50 Hz, 1 mT for 15 min | None | MF exposure upregulated *HSP72*. |
| †Falone et al. 2008 | *Cat, Gpx1*, *Gsr*, *Gstp1*, *Sod1*, and *Sod2* in brain cortex from exposed female Sprague-Dawley rats | 50 Hz, 0.1 mT for 10 d | None | MF exposure downregulated *Gsr*. No effect was observed on *Cat, Gpx1*, *Gstp1*, *Sod1*, or *Sod2* |
| †George et al. 2008 | *Hsp70* in heart tissues from exposed Sprague-Dawley rats | 60 Hz, 0.008 mT for 30 min | Ischemia (after MF exposure) | Pre-exposure to MFs upregulated Hsp70 in rats that underwent ischemia. |
| *Vianale et al. 2008 | *CCL5* in human immortalized HaCaT keratinocytes | 50 Hz, 1 mT for 4, 12, or 72 h | None | *CCL5* was downregulated after 72 h of MF exposure. |
| *De Mattei et al. 2009 | *PTGS2* in bovine synovial fibroblasts | 75 Hz, 1.5 mT for 24 h, pulsed | TNF-$\alpha$ or LPS | Sole MF exposure downregulated *PTGS2*. In addition, MF exposure decreased TNF-$\alpha$- and LPS-induced upregulation. |

Table 4. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| †Mariucci et al. 2010 | *Hsp70* in brain and liver from exposed adult male CD mice | 50 Hz, 1 mT for 15 h/d for 1 d or 7 d | None | No effect |
| †Saadat et al. 2010 | *Gstt1* in testis and liver from exposed male Wistar rats | 50 Hz, 0.5 mT for 30 d | None | No effect |
| *Bouwens et al. 2012 | *PTGS2* in human leukemia THP-1 monocytes and macrophages | 50 Hz, 0.005 mT for 30 min | LPS (during MF exposure) | No effect |
| *Patruno et al. 2012 | *NOS2* in human leukemia THP-1 monocytes | 50 Hz, 1 mT for 24 h | LPS (during MF exposure) | Sole MF exposure upregulated *NOS2*. MF exposure decreases LPS-induced upregulation. |
| †Li et al. 2013 | *Cat* and *Hsp22* in male *Drosophila melanogaster* flies | 50 Hz, 3 mT for 72 h or 312 h | None | MF exposure upregulated H*sp22* and downregulated C*at*. |
| †Villarini et al. 2013 | *Hsp70* in the brain from exposed CD1 mice | 50 Hz, 0.1, 0.2 or 1, 2 mT for 15 h/d for 7 d | None | MF exposure upregulated *Hsp70* in the hippocampus at 0.1mT. No effect was observed in the cerebrum or the cortex. |
| *Wang et al. 2014 | *NFE2L2* in human amniotic epithelial cells | 50 Hz, 1mT, 30 min exposure repeated every 12 h for 21 d, pulsed | OIM (during MF exposure) | Sole MF exposure upregulated *NFE2L2*. In addition, MF exposure increased OIM-induced upregulation. |
| *Fan et al. 2015 | *Tpo* in rat bone marrow mesenchymal stem cells | 50 Hz, 1 mT for 4 h/d for 3 d | None | MF exposure upregulated *Tpo*. |
| *Lee et al. 2015 | *FOXM1* in human MCF-7 breast cancer cells | 60 Hz, 2 mT for 16 h | None | No effect |
| †Liebl et al. 2015 | *Sod1* in a mouse model for ALS (mice expressing mutant Cu/Zn-SOD1) | 50 Hz, 1 mT for 8 or 10 mo | None | No effect |
| *Mahmoudinasab and Saadat 2016 | *NQO1* and *NQO2* in human MCF-7 breast cancer cells | 50 Hz, 0.25 or 0.50 mT for 30 min, continuously or intermittently (5 min on/5 min off, or 15 min on/15 min off) | None | MF exposure with 5 min on/5 min off intermittent exposure downregulated *NQO1* at 0.25 mT (0 h) and upregulated *NQO2* at 0.5mT (0 h and 2 h). Other exposure patterns had no effect. |

Table 4. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Mahmoudinasab et al. 2016 | *CAT*, *GSTM3*, *GSTO1*, *MSGT1*, *MSGT3*, *SOD1* and *SOD2* in human MCF-7 breast cancer cells | 50 Hz, 0.25 or 0.50 mT for 30 min, continuously or intermittently (5 min on/5 min off or 15 min on/15 min off) | None | MF exposure resulted in sporadic and diminutive dysregulations of the assayed genes. |
| †Zhang et al. 2016 | *Hsp22, Hsp26,* and *Hsp70* in *Drosophila melanogaster* flies | 50 Hz, 3 mT for 12 h | Thermal stress (during MF exposure) | MF exposure increased thermal stress-induced upregulation of *Hsp22*, *Hsp26*, and *Hsp70*. |
| *Akbarnejad et al. 2017 | *HMOX1* in human glioblastoma cells | 100 Hz, 10 mT for 120 or 140 h | Temozolomide (during MF exposure) | Sole MF exposure upregulated HMOX1. In addition, MF exposure enhanced temozolomide-induced upregulation. |
| *Aikins et al. 2017 | *MT3* in human bone marrow mesenchymal stem cells | 50 Hz, 1 mT, 12 d | None | MF exposure downregulated *MT3*. |
| *Ehnert et al. 2017 | *CAT*, *GPX1*, *GPX3*, *GSR*, and *SOD2* in human osteoblast | 16 Hz, 0.006 – 0.282 mT for 7 min/d for 3 d, pulsed | None | MF exposure upregulated *CAT*, *GPX3*, *GSR*, and *SOD2*; however, it did not affect *GPX1*. |
| *Kim et al. 2017 | *Nos2* in mouse leukemic macrophage RAW 264.7 cells | 60 Hz, 0.8 mT for 12 h | LPS (during MF exposure) | MF exposure upregulated *Nos2* in LPS-treated cells. |
| *Villarini et al. 2017 | *HSP70* in human neuroblastoma SH-SY5Y cells and SK-N-BE-2 cells | 50 Hz, 0.01, 0.1, or 1 mT for 1 h continuously or 5 h intermittently (15 min on/ 15 min off) | $AlCl_3$ (during MF exposure) | No effect |
| *Lian et al. 2018 | *HSP40*, *HSP70*, *HSP104* in budding yeast | 50 Hz, 6 mT for 4 d | None | No effect |
| *Mahmoudinasab and Saadat 2018a | *CAT*, *GSTM2*, *GSTM3*, *GSTO1*, *GSTP1*, *MGST1*, *MGST3*, *NQO1*, *NQO2*, *SOD1*, and *SOD2* in human MCF-7 breast cancer cells and neuroblastoma SH-SY5Y cells | 50 Hz, 0.5 mT for 24 h, intermittent (15 min on/ 15 min off) | Cisplatin and morphine (during MF exposure) | MF exposure tended to upregulate the assayed genes in the chemically treated cells. This effect was more prominent in SH-SY5Y5 cells than MCF-7 cells. |

Table 4. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Mahmoudinasab and Saadat 2018b | *CAT*, *GSTM2*, *GSTM3*, *GSTO1*, *GSTP1*, *MGST1*, *MGST3*, *NQO1*, *NQO2*, *SOD1*, and *SOD2* in human SH-SY5Y neuroblastoma cells | 50 Hz, 0.5 mT for 30 min, continuously or intermittently (15 min on/15 min off) | β-Lapachone and morphine (during MF exposure) | Intermittent exposure to MFs upregulated *NQO2*, downregulated *GSTM3*, *GSTO1*, *NQO1*, *SOD1*, *MGST3*, *SOD2*, but had no effect on *CAT*, *GSTP1*, *GSTM2*, and *MGST1*. MF effects on the transcription of the assayed genes in the combination of chemical treatment were sporadic and had no apparent pattern. |
| *Costantini et al. 2019 | *HMOX1* and *NOS2* in human gingival cells | 50 Hz, 1 mT for 6 or 18 h, sinusoidal or pulsed (at 12 Hz) | None | MF exposure upregulated *HMOX1* and *NOS2*. *HMOX1* upregulation was higher after 18 h than after 6 h. *NOS2* upregulation was higher after 6 h than after 18 h. |
| *Sun et al. 2019 | *GST* in *Irpex lacteus* fungi | 50 Hz, 3.5 mT for 3 h/d for 4 d | None | MF exposure upregulated *GST*. |
| †Zhou et al. 2019 | *Alox12* in bone tissues from exposed Sprague-Dawley rats | 50 Hz, 1.8 mT for 90 min/d for 2 mo | None | MF exposure upregulated *ALOX12*. |
| ‡Wang et al. 2019 | *Hsp70* in human AC16 cardiomyocytes (*in vitro*), or cardiac tissues from exposed male Sprague-Dawley rats (*in vivo*) | 50 Hz, 0.1 mT. *In vitro*: continuously for 1 h or intermittently for 1.25 h (15 min on/15 min off). *In vivo*: continuously for 7 d | None | No effect |
| *Patruno et al. 2020 | *HMOX1* in human leukemia THP-1 monocytes | 50 Hz, 1 mT for 1, 6, or 24 h | LPS (during MF exposure) | *HMOX1* was upregulated after 6 h and downregulated after 1 h and 24 h in LPS-treated cells. |
| *Vinhas et al. 2020 | *PTGS2* in human tendon-derived cells | 5 Hz, 4 mT for 1 h, pulsed | IL-1β (before MF exposure) | MF exposure downregulated *PTGS2*. In addition, MF exposure decreased IL-1β-induced upregulation of *PTGS2*. |
| †Wang et al. 2020 | *sod-1, sod-2,* and s*od-3* in *Caenorhabditis elegans* worms | 50 Hz, 3 mT, continuous exposure for 15 generations | None | MF exposure upregulated s*od-1, sod-2,* and s*od-3*. |

Table 4. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Consales et al. 2021 | *GCLC, GCLM,* and *SOD1* in differentiating human SH-SY5Y neuroblastoma cells cultured in 3D or traditional 2D cultures | 50 Hz, 1 mT for 72 h | None | MF exposure downregulated *SOD1* only in 3D cultures. |
| *Groiss et al. 2021 | *APOE*, *CAT*, *DHCR24*, *GPX1*, *HSP70*, *PRDX6*, *SOD1*, and *SOD2* in human THP1 leukemic cells or peripheral mononuclear cells (PBMC) | 16.7 Hz, 0.05, 0.25, or 4.8 mT for 1, 3, 6, or 24 h, intermittent (10 min on/10 min off) | LPS (before MF exposure) | MF exposure increased LPS-induced upregulation of *PRDX6*, *DHCR24*, and *SOD2* in TPH1 cells and *HSP70*, *SOD1*, and *SOD2* in PBMC. The increase was mainly in response to the strongest field strength. |

Asterisks* indicate *in vitro* studies, daggers † indicate *in vivo* studies, and double daggers ‡ indicate studies involved both *in vitro* and *in vivo* experiments.
Aluminum chloride ($AlCl_3$), Amyotrophic lateral sclerosis (ALS), Day (d), Hertz (Hz), Hour (h), Interleukin-1β (IL-1β), Lipopolysaccharide (LPS), Magnetic fields (MF), Millitesla (mT), Minute (min), Osteo-induction medium (OIM), Peripheral blood mononuclear cells (PBMC), Pulsed electromagnetic field (PEMF), Tumor necrosis factor-alpha (TNF-α).
Assayed oxidative stress-related genes: 24-Dehydrocholesterol reductase (*DHCR24*), Apolipoprotein E (*APOE*), Arachidonate 12-lipoxygenase (*ALOX12*), Catalase (*CAT*), Chemokine (C-C motif) ligand 5 (*CCL5*), Forkhead box M1 (*FOXM1*), Glutamate-cysteine ligase, catalytic subunit (*GCLC*), Glutamate-cysteine ligase, modifier subunit (*GCLM*), Glutathione peroxidase 1 (*GPX1*), Glutathione peroxidase 3 (*GPX3*), Glutathione reductase (*GSR*), Glutathione S-transferase (*GST*), Glutathione S-transferase mu 2 (*GSTM2*), Glutathione S-transferase mu 3 (*GSTM3*), Glutathione S-transferase omega 1 (*GSTO1*), Glutathione S-transferase pi 1 (*GSTP1*), Glutathione S-Transferase theta 1 (*GSTT1*), Heat shock 16kDa protein (*HSP16*), Heat shock 22kDa protein (*HSP22*), Heat shock 26kDa protein (*HSP26*), Heat shock 27kDa protein (*HSP27*), Heat shock 40kDa protein (*HSP40*), Heat shock 60kDa protein (*HSP60*), Heat shock 70 kDa protein A (*HSP70A*), Heat shock 70 kDa protein B (*HSP70B*), Heat shock 70 kDa protein C (*HSP70C*), Heat shock 70kDa protein (*HSP70*), Heat shock 72kDa protein (*HSP72*), Heat shock 75kDa protein (*HSP75*), Heat shock 78kDa protein (*HSP78*), Heat shock protein 90kDa (HSP90), Heat shock protein 90kDa alpha (HSP90α), Heat shock protein 90kDa beta (HSP90β), Heat shock 104kDa protein (*HSP104*), Heme oxygenase 1 (*HMOX1*), Metallothionein 3 (MT3), Microsomal glutathione S-transferase 1 (*MGST1*), Microsomal glutathione S-transferase 3 (*MGST3*), NAD(P)H dehydrogenase, quinone 1 (*NQO1*), NAD(P)H dehydrogenase, quinone 2 (*NQO2*), Nitric oxide synthase 2, inducible (*NOS2*), Nuclear Factor, Erythroid 2 Like 2 (*NFE2L2*), Peroxiredoxin-6 (*PRDX6*), Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) (*PTGS2*), Superoxide dismutase 1, soluble (*SOD1*), Superoxide dismutase 2, mitochondrial (*SOD2*), Superoxide dismutase 3, extracellular (*SOD3*), Thyroid peroxidase (*TPO*).

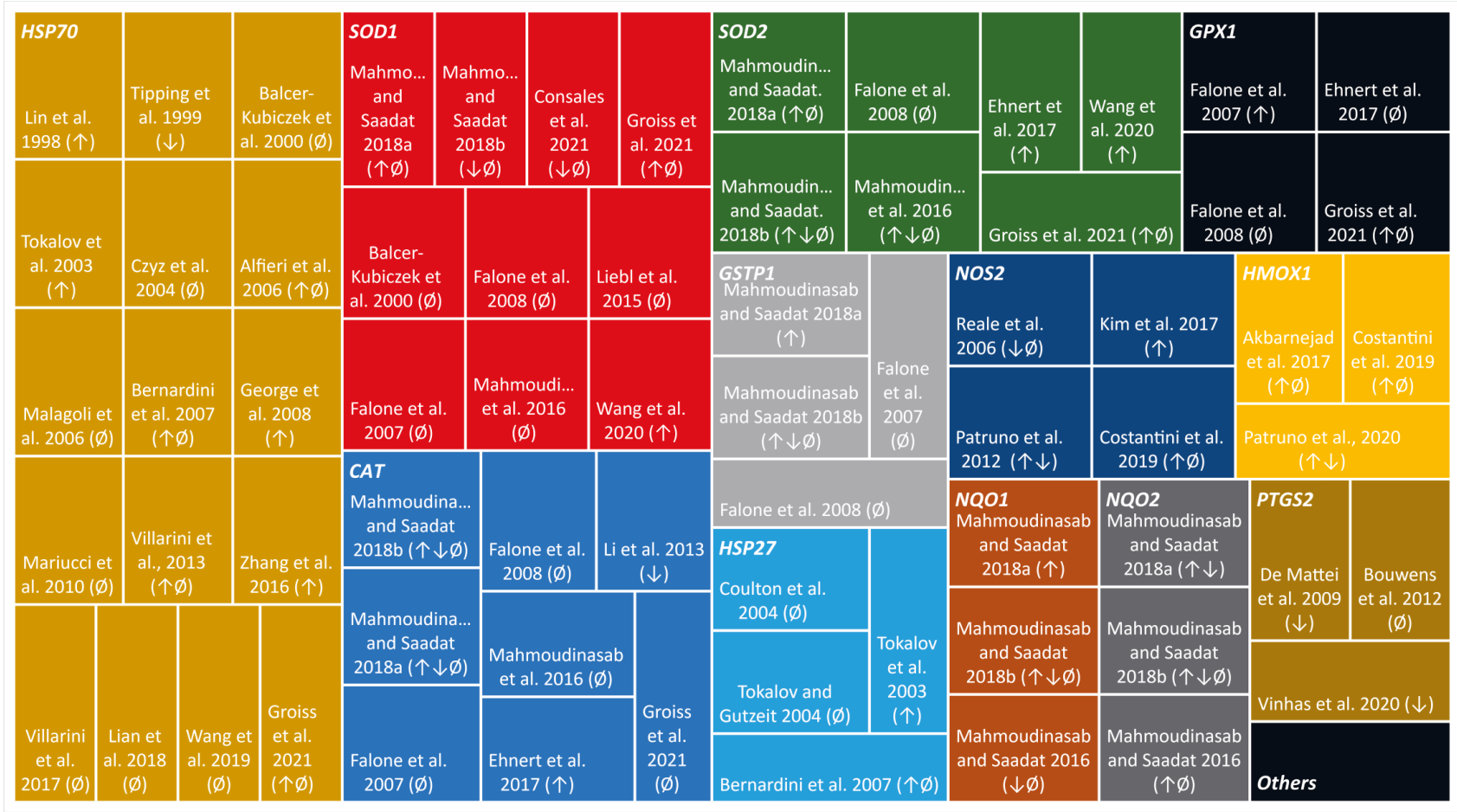


Figure 3. Treemap summarizing studies on ELF MFs effects on the transcription of oxidative stress-related genes. The arrows indicate the directions of the dysregulation (↑: upregulation, ↓downregulation), whereas the symbol Φ indicates no effect. (Online version in color).

## 1.9 SUMMARY OF CIRCADIAN RHYTHM FINDINGDS LINKED TO ELF MFs

The melatonin hypothesis (Stevens, 1987) was one of the first hypotheses suggesting that circadian rhythm disruption could explain possible association between exposure to weak MFs and cancer, primarily breast cancer (the risk of breast cancer was thought to be possibly increased by ELF MFs at that time). The premise of the melatonin hypothesis is that MFs might have an inhibitory effect on melatonin production. Melatonin is a hormone released by the pineal gland and has long been associated with controlling the sleep-wake cycle. Melatonin is a possible oncostatic molecule, and its altered levels are found in many diseases, including cancer (Hill et al., 2011; Jin et al., 2021). In addition to melatonin, cortisol is another universal marker of the circadian rhythm. Cortisol is a hormone released by the adrenal gland and regulated by the central circadian pacemaker in the hypothalamus. Cortisol is believed to play a substantial liaison role between the central and peripheral circadian clocks (Chan and Debono, 2010). In addition, cortisol is suggested to be involved in cancer development via stress-mediated mechanisms (Antonova et al., 2011). The effects of exposure to ELF MFs on melatonin and cortisol levels have been extensively studied, and results were largely contradictory (increased levels, decreased levels, and no effects) (see comprehensive reviews: Touitou and Selmaoui, 2012; Lewczuk et al., 2014; Bouché and McConway, 2019). These contradictory findings were reported in all test systems, including animals and *in vitro* pineal and adrenal glands models. Possible sources of discrepancy in results include differences in exposure parameters and potential interference with confounding factors such as light and variance of circadian phases during the exposures (Lewczuk et al., 2014; Kolbabová et al., 2015).

CRYs are another suggested target of a possible MF action on the circadian system (Lagroye et al., 2011; Vanderstraeten et al., 2015; Juutilainen et al., 2018, Guerra et al., 2019). This suggestion arises from the magnetosensitive properties of the CRYs and their critical involvement in the circadian system (see section 1.5). The evidence for the effects of ELF MFs on cryptochromes is not clear, and studies investigating these effects are few. Fedele et al. (2014) reported that *Drosophila melanogaster* exposed to 3 – 50 Hz MFs exhibited a CRY-dependent increase in locomotor activity and alterations in the circadian period length. In addition, Sherrard et al. (2018) found that the modulation of intracellular levels of ROS after exposure to 10 Hz pulsed MFs was dependent on the presence CRYs in either *Drosophila melanogaster* or immortalized mouse embryonic fibroblasts.

With regards to possible effects on circadian rhythm gene transcription, only two studies investigated the effect of MFs on the transcription of core circadian clock genes (Manzella et al., 2015; Lundberg et al., 2019), and the results were contrasting (Table 5, Fig. 4). Manzella et al. (2015) reported that 50 Hz exposure at 0.1 mT for 1 or 48 h resulted in an alteration in the transcriptional oscillation of *ARNTL*, *CRY1*, *CRY2*, *PER2*, and *PER3* in human dermal fibroblasts. On the other hand, Lundberg et al. (2019) found that 50 Hz exposure at 0.58 mT for 30 min did not affect the transcription of *Cry1*, *Cry2*, and *Per1* in C57BL/6J mice. Besides, few studies investigated the effects of MFs on circadian rhythm-related genes (such as *PRKAR1A*, *EGR1*, *CAMK2G*, *NCOA3*, *NKX2-5*, *TGFB1*, *STAT6*, *RORA*, and *RORC*). These genes are nycthemeral, and their expressions are outputs of the circadian oscillation. From up- and down-regulations to no effects, the impact of MF exposure on these genes was largely inconsistent (Ventura et al., 2005; Girgert et al., 2008; Hong et al., 2012; Nie et al., 2013; Seong et al., 2014; Golbach et al., 2015; Ledda et al. 2018; Costantini et al., 2019; Mahaki et al., 2019; Groiss et al. 2021) (Table 5, Fig. 4). Overall, the evidence for MF effects on circadian rhythm gene transcription is weak, primarily due to the paucity of the conducted investigations.

Table 5. Published studies on the effects of ELF MFs on the transcription of circadian rhythm-related genes.

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| *Ventura et al. 2005 | *Nkx2-5* in mouse embryonic stem cells | 50 Hz, 0.8 mT for 3 or 10 d | None | MF exposure upregulated *Nkx2-5*. |
| *Girgert et al. 2008 | *NCOA3* in human MCF-7 breast cancer cells | 50 Hz, 0.0012 mT for 24, 48, or 96 h | None | Exposure to MFs for 24 h upregulated *NCOA3*. Other exposure durations had no effects. |
| *Hong et al. 2012 | *PRKAR1A* in human MCF-10A normal breast epithelial cells | 60 Hz, 1 mT for 4 or 16 h | None | No effect |
| †Nie et al. 2013 | *Rorc* in PBMC from exposed normal or hepatocarcinoma-bearing female Balb/c mice | 7.5 Hz, 0.4 T for 2 h/d for 30 d | None | No effect |
| *Seong et al. 2014 | *EGR1* in human bone marrow mesenchymal stem cells and mouse ES-derived neural stem cells | 50 Hz, 1 mT for 8 d | None | MF exposure upregulated *EGR1*. |
| *Golbach et al. 2015 | *CAMK2G* in human HL60 and PLB-985 leukemia cells | 50 Hz, 0.005, 0.3, 0.5 mT for 30 min or several days | None | No effect |
| *Manzella et al. 2015 | *ARNTL*, *CRY1, CRY2*, *PER2*, and *PER3* in human dermal fibroblasts | 50 Hz, 0.1 mT for 1 h or 48 h | Serum starvation or serum shock (before MF exposure) | 1-h exposure to MFs induced a circadian oscillation in the transcription of the assayed genes in serum-starved cells. The 48-h continuous exposure to MFs increased the amplitude of the circadian cycle transcription that was previously entrained by serum shock. |
| *Ledda et al. 2018 | *NKX2-5* in human amniotic mesenchymal stromal cells | 7 Hz, 0.0025 mT for 5 d | None | MF exposure upregulated *NKX2-5*. |
| *Costantini et al. 2019 | *TGFB1* in human gingival cells | 50 Hz, 1 mT for 6 or 18 h, sinusoidal or pulsed (at 12 Hz) | None | *TGFB1* was upregulated after 6-h MF exposure. The upregulation caused by sinusoidal MFs was higher than by pulsed MFs. However, downregulation of *TGFB1* was observed after 18-h exposure. |
| †Lundberg et al. 2019 | *Cry1*, *Cry2,* and *Per1* in adrenal glands, liver, hippocampus from exposed male C57BL/6J mice | 50 Hz, 0.58 mT for 30 min | Blue light (during MF exposure) | No effect |

Table 5. Continued

| Reference | Assayed gene(s) | MF exposure | Co-exposure | Response |
|---|---|---|---|---|
| †Mahaki et al. 2019 | *Stat6* and *Rora* in spleen and thymus from exposed male rats | 50 Hz, 0.001 – 2 mT for 2 h/d for 60 d | None | MF exposure downregulated *Rora* (0.001 and 0.1 mT) and *Stat6* (0.1 mT) in the spleen. No effect was found on the thymus. |
| *Groiss et al. 2021 | *EGR1* in human THP1 leukemic cells and human PBMC | 16.7 Hz, 0.05, 0.25, or 4.8 mT for 1, 3, 6, or 24 h, intermittent (10 min on/10 min off) | LPS (before MF exposure) | 3-h exposure to MFs upregulated *EGR1* in THP1 cells. No effect was observed in PBMC. |

Asterisks* indicate *in vitro* studies, and daggers † indicate *in vivo* studies.

Day (d), Hertz (Hz), Hour (h), Millitesla (mT), Minute (min), Peripheral blood mononuclear cells (PBMC).

Assayed circadian rhythm-related genes: Aryl hydrocarbon receptor nuclear translocator-like (*ARNTL*), Calcium/calmodulin-dependent protein kinase II gamma (*CAMK2G*), Cryptochrome 1 (*CRY1*), Cryptochrome 2 (*CRY2*), Early growth response 1 (*EGR1*), Nuclear receptor coactivator 3 (*NCOA3*), NK2 Homeobox 5 (*NKX2-5*), Period circadian regulator 1 (*PER1*), Period circadian regulator 2 (*PER2*), Period circadian regulator 3 (*PER3*), Protein kinase, cAMP-dependent regulatory, type I, alpha (*PRKAR1A*), RAR-related orphan receptor A (*RORA*), RAR-related orphan receptor C (*RORC*), Signal transducer and activator of transcription 6 (*STAT6*), Transforming growth factor, beta 1 (*TGFB1*).

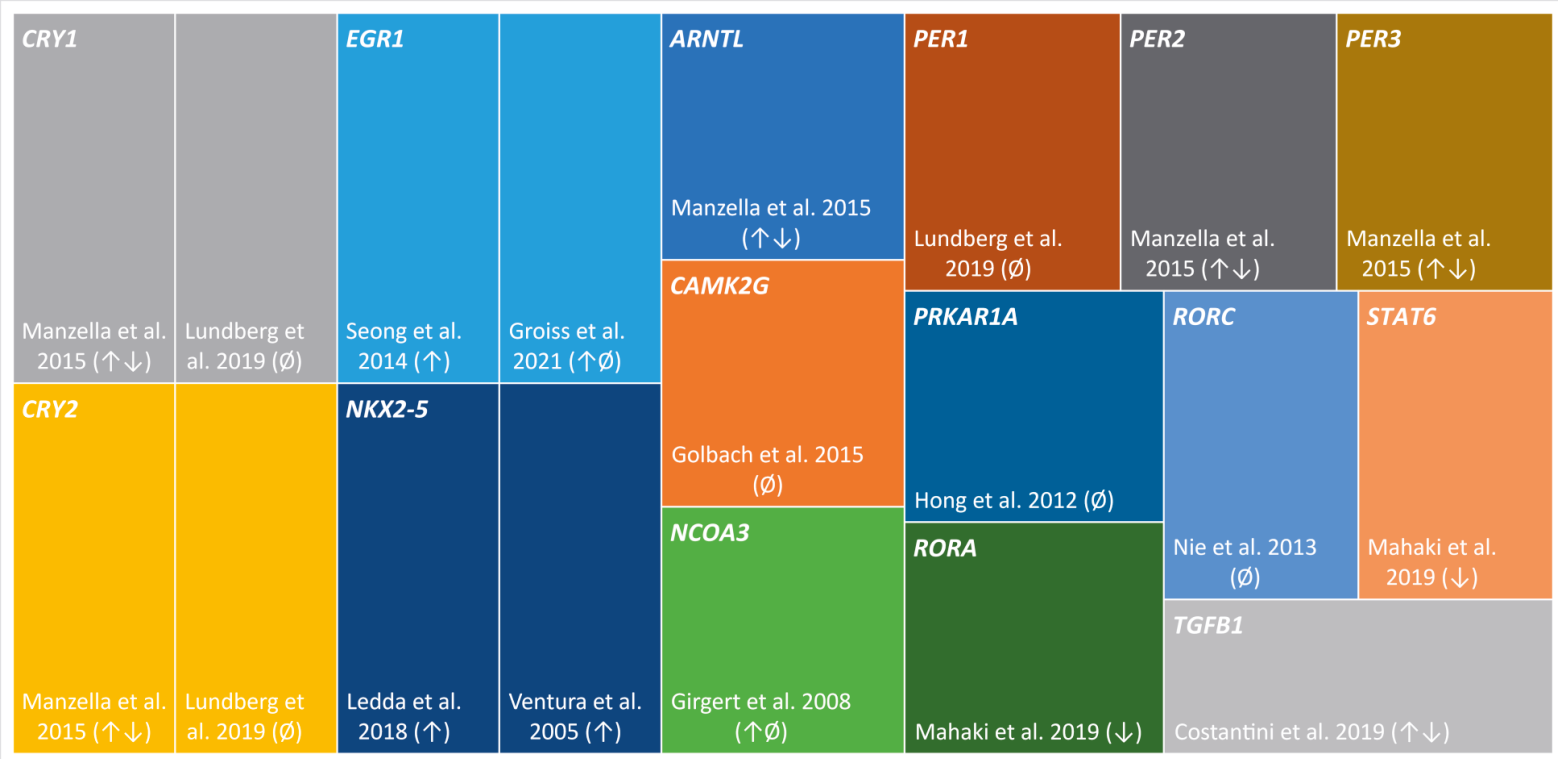


Figure 4. Treemap summarizing the studies on ELF MFs effects the transcription of circadian rhythm-related genes. The arrows indicate the direction of the dysregulation (↑: upregulation, ↓: downregulation), whereas the symbol Φ indicates no effects. (Online version in color).

# 2 AIMS OF THE STUDY

The present study aimed to increase the understanding of the mechanisms of possible carcinogenic effects of RF radiation and ELF MFs. The present study used different types of primary and secondary cells relevant to brain tumors and blood cancer (the two types of cancers epidemiological studies indicated that RF radiation and ELF MFs, respectively, were possibly associated with). In addition to studying the effects of sole exposures to RF fields or ELF MFs, these fields were combined with chemicals known to be genotoxic or oxidative stress-inducing.

More specifically, the present study aimed at answering the following questions:

1. Can RF radiation cause genotoxicity or induce genomic instability?
2. Can ELF MFs cause genotoxicity or modify responses to chemically-induced DNA damage?
3. Can ELF MFs affect ROS production or alter the mRNA levels of oxidative stress-related genes?
4. Can ELF MFs alter the mRNA levels of circadian rhythm-related genes?

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# Chapter 2.

# Assessment of genotoxicity and genomic instability in rat primary astrocytes exposed to 872 MHz radiofrequency radiation and chemicals

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# Chapter 3.

# The duration of exposure to 50 Hz magnetic fields: Influence on circadian genes and DNA damage responses in murine hematopoietic FDC-P1 cells

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# The duration of exposure to 50 Hz magnetic fields: Influence on circadian genes and DNA damage responses in murine hematopoietic FDC-P1 cells

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Gene expression

ABSTRACT

We investigated the effects of 50 Hz extremely low-frequency magnetic fields (MFs) on gene expression related to the circadian rhythm or DNA damage signaling and whether these fields modify DNA damage repair rate after bleomycin treatment. Murine FDC-P1 hematopoietic cells were exposed for different durations (15 min, 2 h, 12 h, and 24 h) to either 200 μT MFs or sham-exposures. Cells were then collected for comet assay or real-time PCR to determine immediate DNA damage level and circadian rhythm gene expression, respectively. To assess DNA-damage signaling and DNA repair rate, the cells were subsequently treated with 20 μg/mL bleomycin for 1 h and then either assayed immediately or allowed to repair their DNA for 1 or 2 h. We found that circadian rhythm-related genes were upregulated after 12 h of MF exposure and downregulated after 24 h of MF exposure, but none of the affected genes were core genes controlling the circadian rhythm. In addition, we found that the repair rate for bleomycin-induced damage was only decreased after MF exposure for 24 h. In conclusion, our findings suggest that the effects of MFs are duration-dependent; they were observed predominantly after long exposures.

## 1. Introduction

Humans are constantly exposed to extremely low-frequency (ELF) magnetic fields (MFs); these fields accompany all forms of electricity use and transmission. The International Agency for Research on Cancer (IARC) has classified ELF MFs as possibly carcinogenic to humans, class 2B [1]. This classification was mainly based on reasonably consistent epidemiological findings suggesting an association between childhood leukemia and residential exposure to weak MFs (above 0.3−0.4 μT). However, evidence for carcinogenic effects from *in vivo* and *in vitro* studies is still inadequate. For these reasons, establishing a generally accepted biophysical mechanism that can explain the possible association between carcinogenicity and MFs remains a high-priority research need.

Circadian rhythm plays a vital role in regulating major cellular activities; anomalies in these activities are associated with cancer development and progression. At the molecular level, circadian rhythm primarily originates from the ~24-h oscillation in the products of eight core circadian clock genes, *Arntl* (also known as *BMAL1*), *Clock*, *Cry1*, *Cry2*, *Csnk1e*, *Per1*, *Per2*, and *Per3*. These genes are organized as an autonomous network and connected by a complex web of feedback loops [2]. Circadian rhythm influences a vast number of genes involved in both cell cycle and DNA damage responses [3,4]. More specifically, the circadian clock gates cells' entry into S-phase, where DNA replicates, and gates the transition between $G_2$-phase and M-phase, in which the cell mitotically divides [3]. The proper function of this cell-cycle control is central for preventing carcinogenicity-related effects: cell-cycle arrest allows for both the repair of possible DNA damage and for apoptosis to remove the harmful cells [5].

Previously, we hypothesized a link between cancer and exposure to environmental MFs [6]. The hypothesis proposes that the interaction between MFs and radical reactions in cryptochromes, magneto-sensitive flavoproteins, could disrupt circadian rhythm and alter the cell cycle. In turn, these changes could result in abnormal responses to DNA damage, the accumulation and persistence of mutations, and, eventually, cancer. Here, in murine hematopoietic FDC-P1 cells, we explored the expression of the eight core circadian genes and other 76 genes that are associated with circadian rhythm and contribute to a wide range of biological processes. Potential effects were investigated in response to short (15 min), moderate (2 h), and long (12 h and 24 h) exposures to clarify whether different durations of MF exposure affect divergently circadian rhythm or DNA damage responses. To assess these responses, we

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explored whether exposure to MF alone could damage DNA and whether pre-exposure to MFs could affect the level of DNA damage induced by bleomycin, a DNA strand-breaking chemical. In addition, we examined the possibilities that MFs might modify the rate of DNA repair after bleomycin-induced DNA damage or the expression of genes involved in DNA damage signaling. The experiments were performed using 50 Hz ELF MFs at 200 μT magnetic flux density, which is the reference level for general public exposure recommended by the International Commission on Non-Ionizing Radiation Protection [7].

## 2. Materials and methods

### 2.1. Reagents

Bleomycin (Cayman chemical company, Ann Arbor, Michigan, USA); D-glucose (ICN Biomedicals, USA); Ethanol (Altia, Rajamäki, Finland); Ethidium bromide (Sigma, USA); Ethylenediaminetetraacetic acid (EDTA) (Merck, Netherlands); Fetal bovine serum (FBS) (Gibco, South America); Low melting point agarose (LMPA) (Sigma, USA); N-Lauroylsarcosine sodium salt (SDS) (Sigma, UK); Normal melting point agarose (NMPA) (Bio Whittaker Molecular Application, Rockland, Maine, USA); Penicillin/streptomycin antibiotic solution (Gibco, USA); Potassium chloride (KCl) (Merck, Germany); Potassium dihydrogen phosphate ($KH_2PO_4$) (Merck, Germany); Sodium bicarbonate ($NaHCO_3$) (Riedel-de Haën, Seelze, Germany); Sodium chloride (NaCl) (Fisher Scientific, UK); Sodium hydrogen phosphate ($Na_2HPO_4$) (Merck, Germany); Sodium hydroxide (NaOH) (VWR Chemicals Prolabo, Czech Republic); Tris(hydroxymethyl)aminomethane (TRIS) (Sigma, USA); Triton x-100 (DOW chemicals, Midland, Michigan, USA).

### 2.2. Cell culture

We conducted experiments using factor-dependent continuous - Paterson 1 cells (FDC-P1) (ATCC® CRL-12103™). These cells originate from the bone marrow of *Mus musculus* mice (DBA/2). FDC-P1 cells are interleukin 3-dependent myeloid progenitor cells that differentiate to monocytes, the largest type of leukocytes (therefore, these cells are relevant for studying a mechanism on how MFs could cause childhood leukemia) [8]. Cells were grown in Dulbecco's modified Eagle medium (Gibco, Paisley, UK) containing 4.5 g/l glucose. The medium was supplemented with 10 % (V/V) heat-inactivated FBS, 50 U/mL penicillin, 50 μg/mL streptomycin, and 10 % (V/V) interleukin 3-containing conditioned medium. The conditioned medium was collected by filtering the culture media of WEHI-3 cells (ATCC® TIB-68™). FDC-P1 cells' doubling time, ~11 h, was analyzed by counting cells using Bürker chambers and the Moxi Z automated cell counter (Orflo Technologies, Ketchum, Idaho, USA). Cells were subcultured every other day and maintained at a density between $1 \times 10^5$ and $1 \times 10^6$ cells/mL. For experiments, we used cells from passage numbers 5–15. Cells were plated 24 h before exposure in 24-well plates (Nunclon™ Delta Surface, Thermo Fisher Scientific, Roskilde, Denmark). Each cultured well in a plate contained $1 \times 10^5$ cells in 1 mL of complete medium.

### 2.3. Sham and MF exposure

The setup of the exposure system is presented in Fig. 1A. MF and sham exposures were done in two identical temperature-controlled and atmosphere-regulated cell culture incubators (+37 °C, 5% $CO_2$; Panasonic MCO-170AICUV, Panasonic Healthcare Co., Japan). The incubators housed two identical coil systems that produce a horizontal MF. Each coil system was made of a cuboid graphite rack with inner dimensions of 36 cm height ×36 cm width ×26 cm depth (Fig. 1B). Each rack contained three coils, 13 cm apart from each other. The outermost coils had twelve turns of copper wire (1.5 mm diameter) each, while the coil in the middle had five turns. The coils were connected in series with two 1 Ω (tolerance ± 5%) resistors (Sfernice RPS500 DH).

A 50 Hz sinusoidal signal was generated using a B&K Precision 5 MHz function/arbitrary 4052 waveform generator (B&K Precision, Yorba Linda, California, USA) and amplified using a Europower EP4000 power amplifier (Behringer, Willich, Germany). During the experiments, the magnetic flux density was set to 200 μT. Magnetic flux density was monitored using a TM-192 triaxial magnetic field meter (Tenmars Electronics Co., Taipei, Taiwan). The 24-well plates were positioned in the center of the coil system to guarantee either a uniform magnetic flux density or identical sham exposure conditions. The heterogeneity of the field in the area that contained the plates during exposures was < 2 % (measured using a Hirst single-axis PA1889 axial probe connected to a Hirst GM08 Gaussmeter, Hirst Magnetic Instruments Ltd, Cornwall, UK).

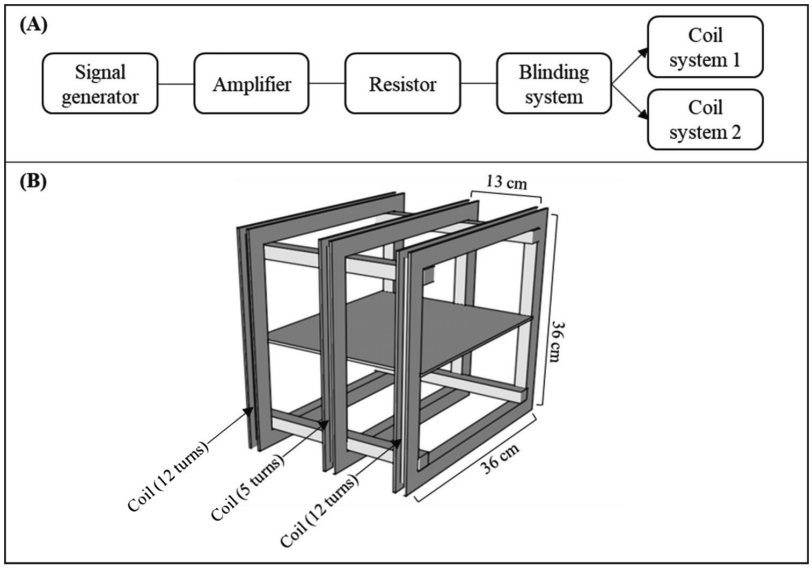


**Fig. 1.** (A) Presentation of the exposure system setup and (B) Sketch diagram of the coil system.

In the incubators, the background low-frequency magnetic flux density was < 2 μT. The static geomagnetic field had a magnetic flux density of ~30 μT (measured using a Hirst single-axis fluxgate AFG100 axial probe connected to a Hirst GM08 Gaussmeter). Fluke 52 K/J Thermometer (Fluke Co., Everett, WA, USA) indicated no differences in the temperatures between the two incubators upon applying the current. No mechanical vibrations were detected using a B&K 4366 accelerometer (Brüel & Kjær, Nærum, Denmark) connected to Wärtsilä 7178D sound level meter (Wärtsilä, Helsinki, Finland).

The exposure system also had a computerized blinding system built at our university. With each new exposure, the blinding software randomly applied current to only one of the two coil systems, and the other one served as a coil system for sham exposure. These random applications were automatically recorded and revealed only after endpoint measurement and data acquisition.

### 2.4. Experimental protocol

Fig. 2 is a graphical presentation of the used schedules for sham and MF exposures and chemical treatments. Cells were exposed to either MFs or sham exposure for 15 min (short duration), 2 h (moderate duration), 12 h (long duration, about one doubling time of FDC-P1 cells), or 24 h (long duration, about two doubling times). After exposure, cells were snap-frozen in liquid nitrogen and stored at −80 °C for the later real-time quantitative polymerase chain reaction (RT-qPCR) to assay the expression level of the eight core circadian clock genes and other 76 circadian rhythm related genes (Supplementary Table 1).

For studying MFs' effects on the level of DNA damage and DNA damage repair rate, exposed cells were either cultivated in fresh medium and immediately assayed for DNA damage *via* comet assay or chemically treated by bleomycin at a concentration of 20 μg/mL for 1 h. We used this bleomycin concentration and this relatively short bleomycin treatment duration guided by the previous studies that assayed the bleomycin-induced DNA damage *via* comet assay [9,10] and by our preliminary experiments. After this, the bleomycin-treated cells were either immediately assayed for DNA damage or allowed to repair their DNA for 1 h or 2 h before the assay.

When analyzing the expression of 84 DNA damage-signaling involved genes (Supplementary Table 2), attention was given to the 24-h exposure duration because MFs significantly impaired the bleomycin-induced DNA damage repair rate only after this exposure duration. Thus, in DNA damage-signaling gene expression experiments, no 2-h exposure duration was applied. Instead, additional timepoints of bleomycin treatment for 15 min and 30 min were introduced after the 24-h exposure duration. Moreover, the repair of bleomycin-induced DNA damage was allowed for 2 h in 12-h exposed cells, but for 1 h or 2 h in 24-h exposed cells.

### 2.5. RT-qPCR

RNA extraction from the coded frozen-cell samples was carried out using the RNeasy® Mini Kit (QIAGEN, Hilden, Germany). The extracted RNA was examined for quantity and purity using an ultraviolet-visible spectrophotometer (NanoDrop® ND-1000, Thermo Fisher Scientific, Waltham, USA). Complementary DNA (cDNA) synthesis was carried out according to RT$^2$ First Strand Kit instructions (QIAGEN Sciences, Maryland, USA). For gene expression assay, cDNA was stained with RT$^{2®}$ SYBR Green qPCR Mastermix (QIAGEN Sciences, Maryland, USA). The cycling program of LightCycler® 480 II (Roche Diagnostics, Basel, Switzerland) was set according to the RT$^2$ Profiler™ PCR Array Handbook (QIAGEN Sciences, Maryland, USA).

The LightCycler® 480 Software (Version 1.5, Roche Diagnostics, Basel, Switzerland) defined a crossing point ($C_p$) for each gene. $C_p$ values were normalized to the average expression level of 5 housekeeping genes: *Actb*, *B2m*, *Gapdh*, *Gusb*, and *Hsp90ab1*. For exploring MFs' effect on gene expression, the $2^{-\Delta\Delta Cp}$ method was used to calculate MF/sham fold changes. We have reported all the statistically significant fold changes since the size of the MFs' effect is generally rather small [1,6].

### 2.6. Comet assay

We used comet assay, also known as single-cell electrophoresis assay, to assess DNA damage level and DNA damage repair rate. Comet assay was performed under alkaline conditions (electrophoresis buffer pH > 13) to detect and quantify single- and double-strand breaks [11]. At the beginning of the assay, cells were diluted in Hank's balanced salt

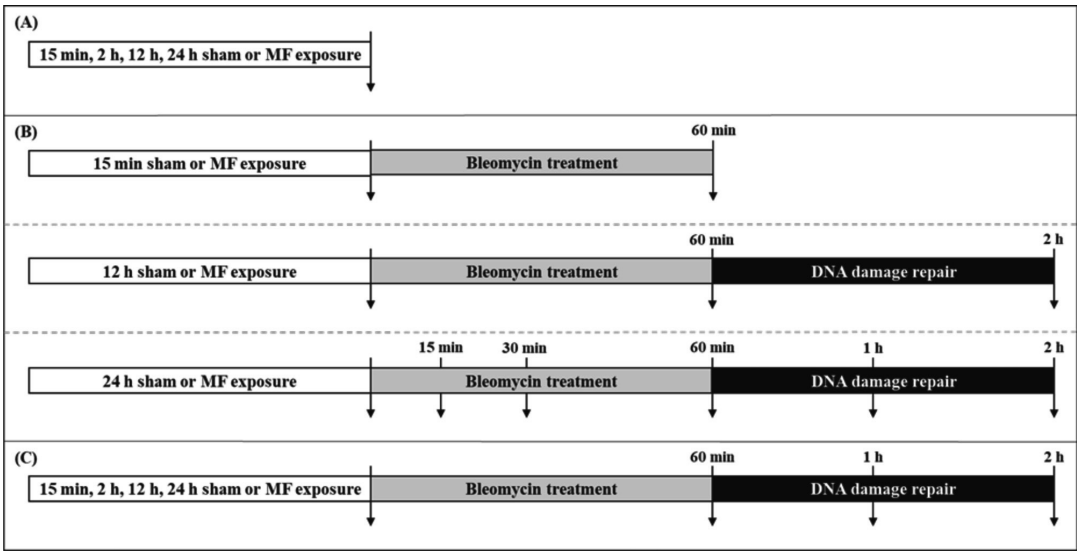


**Fig. 2.** Schedules for sham and MF exposures and bleomycin treatments. Experiments to determine (A) Circadian rhythm gene expression, (B) Gene expression related to DNA damage signaling, and (C) DNA damage and DNA damage repair. Arrows (↓) represent the time points at which the samples were collected.

**Table 1**
Genes related to circadian rhythm that were dysregulated by exposure to MFs for different durations. Mean fold changes (MF/sham) ± SEM from three independent experiments are presented for each gene.

| MF | Downregulated genes | Upregulated genes |
|---|---|---|
| 15 min | *Arntl2* 0.540 ± 0.012** | *Chrnb2* 1.448 ± 0.034*<br>*Csnk2a2* 1.116 ± 0.028* |
| 2 h | – | – |
| 12 h | – | *Bhlhe40* 1.229 ± 0.048*, *Csnk1d* 1.172 ± 0.03*<br>*Esrra* 1.509 ± 0.057**, *Fbxl3* 1.240 ± 0.048*<br>*Mapk1* 1.291 ± 0.034*, *Mapk14* 1.192 ± 0.045*<br>*Mapk3* 1.286 ± 0.04*, *Nfil3* 1.194 ± 0.046*<br>*Prkaca* 1.153 ± 0.01*, *Prkacb* 1.178 ± 0.039*<br>*Prkar2b* 1.331 ± 0.042*, *Slc9a3* 2.875 ± 0.484*<br>*Smad4* 1.090 ± 0.013*, *Stat5a* 1.095 ± 0.007**<br>*Timeless* 1.103 ± 0.018*, *Wee1* 1.181 ± 0.043* |
| 24 h | *Camk2b* 0.762 ± 0.019*<br>*Csnk1d* 0.914 ± 0.006**<br>*Ppargc1a* 0.970 ± 0.004*<br>*Prkar2a* 0.922 ± 0.002**<br>*Rora* 0.841 ± 0.01*<br>*Slc9a3* 0.792 ± 0.042* | – |

* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

solution (5.6 mM D-glucose, 5.3 mM KCl, 0.4 mM $KH_2PO_4$, 4 mM $NaHCO_3$, 137 mM NaCl, 0.3 mM $Na_2HPO_4$) and centrifuged (200 *g*, 5 min, 4 °C). Cells were resuspended in phosphate-buffered saline (2.7 mM KCl, 1.5 mM $KH_2PO_4$, 137 mM NaCl, 8.1 mM $Na_2HPO_4$) to a final concentration of $1 \times 10^6$ cells/mL. Approximately $1.5 \times 10^5$ cells (15 μL of the suspension) were embedded into 75 μL 0.5 % LMPA, spread on microscopic slides pre-coated with a thin layer of 1% NMPA, covered with a coverslip, and placed on ice until the agarose solidified (at least 5 min), then coverslips were removed. Slides were immersed in the lysis buffer (100 mM EDTA, 1% SDS, 2.5 M NaCl, 10 mM TRIS, and 1% Triton x-100) to be kept in the dark at 4 °C for 1 h. Subsequently, slides were placed into a covered tank containing the electrophoresis buffer (1 mM EDTA and 300 mM NaOH) for 25 min at ambient temperature to unwind DNA. Electrophoresis was run for 45 min at 24 V (0.6 V/cm) and 380 mA. Slides were then transferred in a dark box for neutralization (0.4 M TRIS buffer, pH 7.5, 3 × 5 min) and fixation (96 % ethanol, 1 min).

**Table 2**
Genes related to DNA damage signaling that were dysregulated after exposure to MFs upon different experimental conditions. Mean fold changes (MF/sham) ± SEM from three independent experiments are presented for each gene.

| Exposure parameters<br>MF | Bleomycin | DNA repair | Downregulated genes | Upregulated genes |
|---|---|---|---|---|
| 15 min | w/o | n/a | *Msh2* 0.865 ± 0.025* | *Prkdc* 1.144 ± 0.029* |
| | 60 min | n/a | *Exo1* 0.964 ± 0.008* | – |
| 12 h | w/o | n/a | *Ercc1* 0.967 ± 0.007*<br>*Fancc* 0.932 ± 0.014* | – |
| | 60 min | n/a | *Xrcc1* 0.974 ± 0.04* | *Brca1* 1.023 ± 0.003*<br>*Nthl1* 1.052 ± 0.004**<br>*Topbp1* 1.047 ± 0.009* |
| | | 2 h | – | *Mif* 1.073 ± 0.01* |
| 24 h | w/o | n/a | *Blm* 0.965 ± 0.006*<br>*Cdc25a* 0.930 ± 0.004**<br>*Rad9a* 0.938 ± 0.007*<br>*Xrcc2* 0.913 ± 0.012* | *Nbn* 1.114 ± 0.023* |
| | 15 min | n/a | *Brip1* 0.851 ± 0.018*<br>*Pttg1* 0.870 ± 0.007** | *Ercc1* 1.117 ± 0.018*<br>*Rad9a* 1.128 ± 0.024*<br>*Ung* 1.168 ± 0.034* |
| | 30 min | n/a | – | – |
| | 60 min | n/a | – | *Atrx* 1.074 ± 0.014*<br>*Bax* 1.071 ± 0.009*<br>*Pole* 1.074 ± 0.009*<br>*Rad50* 1.112 ± 0.02* |
| | | 1 h | *Mbd4* 0.878 ± 0.024*<br>*Pttg1* 0.880 ± 0.017* | *Atrx* 1.053 ± 0.009*<br>*Mcph1* 1.063 ± 0.009*<br>*Mdc1* 1.046 ± 0.007* |
| | | 2 h | – | *Hus1* 1.039 ± 0.009*<br>*Rad51c* 1.051 ± 0.007*<br>*Smc3* 1.066 ± 0.01* |

* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

For the analysis, slides were coded and stained with 20 μg/mL ethidium bromide. Fluorescent visualization of the nuclei was done by Carl Zeiss AxioImager A1 microscope (Axio Imager A1, Carl Zeiss, Göttingen, Germany). One hundred nuclei were analyzed per sample. The analysis was performed using Comet assay IV image analysis software (Perceptive Instruments, Haverhill, UK). Olive tail moment (OTM; a measure of tail length × a measure of DNA in the tail) was used as a DNA damage parameter.

## 3. Statistical analyses

We used two-way and three-way analyses of variance in the IBM® SPSS® Statistics package (version 25, SPSS Inc, Chicago, Illinois, USA) to statistically analyze gene expression level, DNA damage level, and the repair rate of DNA damage. In these analyses, the replicate effect was considered a random factor. MF exposure and DNA damage repair times were regarded as fixed factors, and $\Delta C_p$ or the normalized OTM was set as the dependent variable. MF-exposed and sham-exposed cells were compared pairwise using Fisher's Least Significant Difference (LSD) post-hoc analysis. All results are from three independent experiments. A p-value of less than 0.05 was considered statistically significant.

## 4. Results

### 4.1. Circadian rhythm-related genes

Compared to sham exposure, MF exposure resulted in 25 statistically significant dysregulation events in 23 circadian rhythm-related genes (Table 1). However, none of these genes was a core gene for circadian rhythm. There were 7 downregulations and 18 upregulations. In response to 15-min MF exposure, 1 gene was downregulated, and 2 genes were upregulated. MF exposure for 2 h did not affect gene expression levels. However, interestingly, a total of 16 genes were upregulated after 12 h of MF exposure. The effect of 24-h MF exposure was in the opposite direction; the 6 affected genes were all downregulated. Two of the dysregulated genes, *Csnk1d* and *Slc9a3*, showed altered expression levels after both 12 h and 24 h of MF exposure.

### 4.2. DNA damage signaling-related genes

Affected genes related to DNA damage signaling were scattered across all MF exposure durations and time points of bleomycin treatment and DNA damage repair (Table 2). Despite various experimental settings, MF exposures resulted only in 32 statistically significant dysregulation events in 28 genes compared to sham exposure. There were 13 downregulations and 19 upregulations. Four of the dysregulated genes, *Atrx*, *Ercc1*, *Pttg1*, and *Rad9a,* showed altered expression levels after different experimental settings.

### 4.3. DNA damage level and DNA damage repair rate

Pre-exposure to MF did not affect the level of DNA damage immediately after bleomycin treatment compared to bleomycin treatment alone (Fig. 3A: 15 min MF ($p = 0.781$), Fig. 3B: 2 h MF ($p = 0.556$), Fig. 3C: 12 h MF ($p = 0.051$), Fig. 3D: 24 h MF ($p = 0.752$)). However, pre-exposure to MF for 24 h significantly decreased the repair rate of bleomycin-induced DNA damage (Fig. 3D, $p = 0.025$). In addition, 24-h MF pre-exposure increased the level of bleomycin-induced DNA damage after 2 h of repair time (Fig. 3D, $p < 0.001$). Such an effect was not seen for other exposure durations. Importantly, exposure to MFs alone affected neither DNA damage levels nor DNA damage repair rate (Fig. 3 A, B, C, and D).

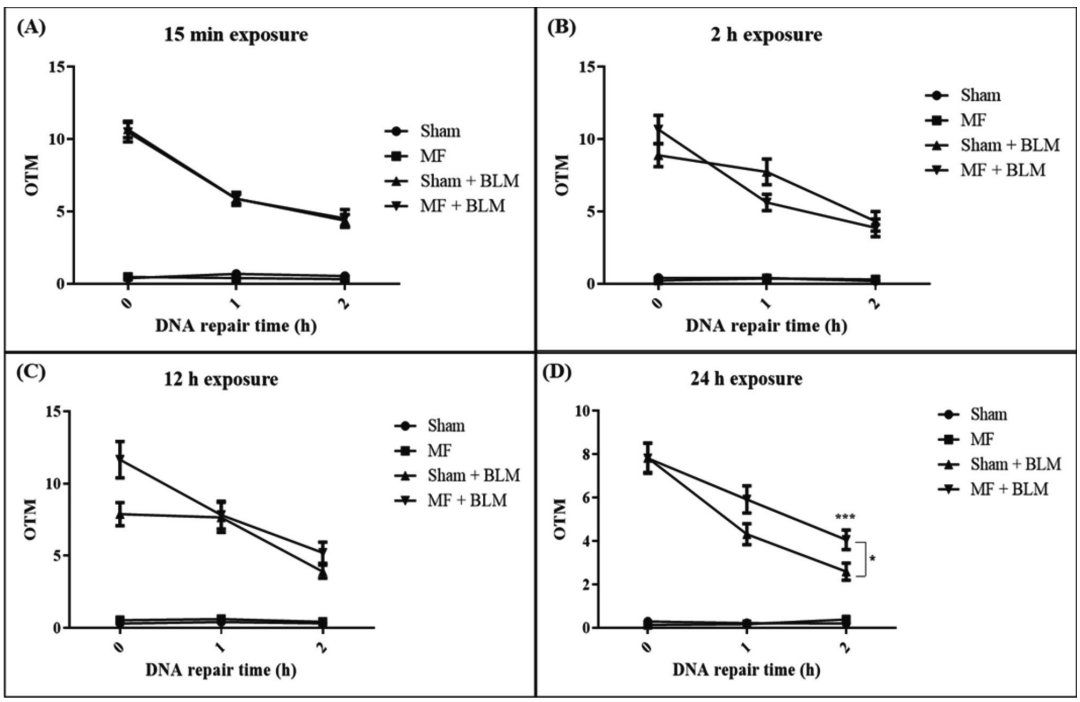


**Fig. 3.** Levels of DNA damage and repair rates in FDCP-1 cells after exposure to MFs or sham exposure for (A) 15 min, (B) 2 h, (C) 12 h, and (D) 24 h, with or without bleomycin (BLM) treatment. OTMs represent the mean of 300 nuclei; each error bars indicate SEM from three independent experiments. Thus, 100 nuclei were scored per sample in each experiment. The symbol] represents the overall effect of MF exposure. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

## 5. Discussion

Here, we investigated the effects of ELF MF exposure duration on the expressions of genes related to circadian rhythm and DNA damage signaling, in addition to evaluating whether DNA damage responses were modified in FDC-P1 cells. The results indicate that the duration of MF exposure was a decisive factor for the effects observed in the DNA repair rate and the expression of genes related to circadian rhythm (but not in core circadian rhythm genes). However, only sporadic MF effects were found in genes related to DNA damage signaling.

An important finding of this study was that the expressions of circadian rhythm-related genes were affected differently by the applied MF exposure durations (15 min., 2 h, 12 h, and 24 h). While only three genes showed dysregulation after MF exposure for up to 2 h, most of the alterations in expressions occurred following 12 h and 24 h of exposure. The changes induced after the 12-h exposure were consistent; they were all upregulations. The effect of 12-h MF exposure did not seem to be persistent; the upregulations caused by 12-h MF exposure were not detected after 24-h MF exposure. On the other hand, the effect of 24-h MF exposure was in the opposite direction; the changes caused by 24-h MF exposure were all downregulations. This pattern of up- then downregulation responding to extending MF exposure duration indicates a systematic shift in gene expression levels. Interestingly, although the expressions of these circadian-rhythm genes were affected in a patterned manner after 12 h and 24 h of MF exposures, none of the eight core genes were affected by any of the four exposure durations. This suggests that the observed changes after 12 h and 24 h of MF exposures are not likely to have originated from the circadian rhythm itself (the core genes regulate it). Therefore, we investigated the affected circadian rhythm-related genes using the GenBank® (www.ncbi.nlm.nih.gov/genbank) and GeneCards® (www.genecards.org) online platforms and noted that many of these genes are also involved in other cellular processes such as apoptosis, cell differentiation, and oxidative stress. In particular, we identified several genes involved in oxidative stress, *e.g.*, *Arntl2*, *Chrnb2*, *Mapk14*, *Smad4*, *Rora*, and Slc*9a3* (NHE3). The possible role of oxidative stress in our observed changes is also supported by the finding that *Slc9a3*, the most affected gene after both 12 h and 24 h of MF exposure, has been shown to be involved in mitochondrial-mediated oxidative stress [12]. As *Slc9a3* gene expression was upregulated after 12-h MF exposure and downregulated after 24-h exposure, this possibly indicates a shift in the mitochondrial oxidative status. Taken together, our results suggest the involvement of other events than circadian rhythm disruption in MF-induced effects.

Interestingly, MF-exposure duration was also a key player in the repair rate of bleomycin-induced DNA damage. Our results demonstrated that the longest MF-exposure duration (24 h) decreased the rate of DNA repair. We did not observe any effects on repair rates for shorter exposures (15 min., 2 h, and 12 h). Although the majority of the previous studies have not reported any effects of MFs on DNA repair (*e.g.*, [13–15]), two studies [16,17] have shown that MFs can modify the rate of repair after chemically induced DNA damage in mammalian cells. In these two studies (similarly to the present one), the duration of MF exposure was 24 h, and it preceded the chemical treatment. Noticeably, the direction of the MF effect contradicts in these previous studies. Robison et al. [16] found MFs to decrease the repair rate of hydrogen peroxide-induced DNA damage in HL-60 cells and HL-60R cells, and Luukkonen et al. [17] reported an enhancement of menadione-induced DNA damage repair rate in human neuroblastoma SH-SY5Y cells. While the present results support the findings of Robison et al. [16], the differences in used co-exposure agents, MF flux densities, and cells between the studies may account for the observed differences.

The MF-induced changes in the expressions of genes related to DNA damage signaling were generally of small size and sporadic. At first glance, there is an apparent controversy in our findings: no systematic MF effects on DNA-damage signaling genes were observed, but a decreased DNA repair rate of bleomycin-induced damage by 24-h MF exposure was found. However, individual gene-expression changes may explain this controversy. Indeed, all three genes (*Atrx*, *Pttg1*, and *Rad9a*) that were affected more than once under the different experimental conditions of 24-h MF exposure are explicitly related to bleomycin-induced DNA damage [18–20]. Although we carefully selected the sample-collection times based on the literature (*e.g.* [21,22],), another possible explanation for this controversy is that these time points were inadequate for detecting maximal gene expression-level: several of the DNA-damage responses are relatively rapid, and their expression levels oscillate quickly [21]. In addition to the present findings, these oscillations may also explain, in part, controversies in the current literature concerning gene- and protein-level responses to MFs [23–26]. It is also important to note that, in general, our gene expression-level findings (both for DNA damage signaling- and for circadian rhythm-related genes) were of small in magnitude. Thus, the biological significance of the current findings needs to be clarified by further studies.

In the present study, we evaluated important steps of our previously suggested hypothesis for environmental magnetocarcinogenesis. This hypothesis [6] proposes that the MF-cryptochromes interaction could disrupt circadian rhythm, alter the cell cycle, and in turn, could impair DNA damage responses. Other research groups have also hypothesized cryptochromes to be target molecules for MF interactions [27,28]. However, here we found no impact of MFs on the cryptochrome-encoding genes, *Cry1* and *Cry2*. Therefore, concerning experimental studies on mammalian models, our present findings support the previous *in vivo* results [29] but are inconsistent with earlier *in vitro* findings on altered *Cry1* and *Cry2* expression levels by MFs [30]. It should be noted that the above studies vary significantly in experimental protocols and the biological models used (compared to each other and the present study). Thus, definite conclusions are not yet possible. However, as we did not observe any MF effects on the expressions of *Cry1* and *Cry2* (or other core circadian-rhythm genes), our results point to a direction that other mechanisms than circadian rhythm disruption are involved in possible carcinogenic effects of MFs.

## 6. Conclusion

In the present study, exposure to MFs did not affect the expression levels of core circadian-rhythm genes, suggesting that cellular responses to MFs do not originate from the circadian rhythm itself. No systematic effects of MFs were observed at any of the analyzed endpoints after 15 min and 2 h exposures. However, exposure to MFs for 12 h and 24 h caused patterned responses in circadian rhythm-related genes. In addition, the 24-h MF exposure decreased the repair rate of bleomycin-induced DNA damage. Taken together, the present results indicate that MF exposure duration is a crucial factor for eliciting biological responses in murine FDC-P1 hematopoietic cells.

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## CRediT authorship contribution statement

**Ehab Mustafa:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Jukka Luukkonen:** Conceptualization, Supervision, Writing - review & editing. **Jenny Makkonen:** Methodology, Software, Writing - review & editing. **Jonne Naarala:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mrfmmm.2021.111756.

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**Online Resources**

Supplementary Table 1: Mouse circadian rhythm genes. PAMM-153ZF RT2 Profiler PCR Array (QIAGEN Sciences, Maryland, USA)

| Gene | Accession number | Description |
|---|---|---|
| *Aanat* | NM_009591 | Arylalkylamine N-acetyltransferase |
| *Alas1* | NM_020559 | Aminolevulinic acid synthase 1 |
| *Arntl* | NM_007489 | Aryl hydrocarbon receptor nuclear translocator-like |
| *Arntl2* | NM_172309 | Aryl hydrocarbon receptor nuclear translocator-like 2 |
| *Bhlhe40* | NM_011498 | Basic helix-loop-helix family, member e40 |
| *Bhlhe41* | NM_024469 | Basic helix-loop-helix family, member e41 |
| *Camk2a* | NM_177407 | Calcium/calmodulin-dependent protein kinase II alpha |
| *Camk2b* | NM_007595 | Calcium/calmodulin-dependent protein kinase II, beta |
| *Camk2d* | NM_023813 | Calcium/calmodulin-dependent protein kinase II, delta |
| *Camk2g* | NM_178597 | Calcium/calmodulin-dependent protein kinase II gamma |
| *Cartpt* | NM_013732 | CART prepropeptide |
| *Noct* | NM_009834 | CCR4 carbon catabolite repression 4-like (S. cerevisiae) |
| *Chrnb2* | NM_009602 | Cholinergic receptor, nicotinic, beta polypeptide 2 (neuronal) |
| *Clock* | NM_007715 | Circadian locomotor output cycles kaput |
| *Creb1* | NM_133828 | CAMP responsive element binding protein 1 |
| *Creb3* | NM_013497 | CAMP responsive element binding protein 3 |
| *Crx* | NM_007770 | Cone-rod homeobox containing gene |
| *Cry1* | NM_007771 | Cryptochrome 1 (photolyase-like) |
| *Cry2* | NM_009963 | Cryptochrome 2 (photolyase-like) |
| *Csnk1a1* | NM_146087 | Casein kinase 1, alpha 1 |
| *Csnk1d* | NM_139059 | Casein kinase 1, delta |
| *Csnk1e* | NM_013767 | Casein kinase 1, epsilon |
| *Csnk2a1* | NM_007788 | Casein kinase 2, alpha 1 polypeptide |
| *Csnk2a2* | NM_009974 | Casein kinase 2, alpha prime polypeptide |
| *Dbp* | NM_016974 | D site albumin promoter binding protein |
| *Egr1* | NM_007913 | Early growth response 1 |
| *Egr3* | NM_018781 | Early growth response 3 |
| *Epo* | NM_001312875 | Erythropoietin |
| *Esrra* | NM_007953 | Estrogen related receptor, alpha |
| *Fbxl21* | NM_178674 | F-box and leucine-rich repeat protein 21 |

Supplementary Table 1. Continued

| Gene | Accession number | Description |
|---|---|---|
| *Fbxl3* | NM_015822 | F-box and leucine-rich repeat protein 3 |
| *Hebp1* | NM_013546 | Heme binding protein 1 |
| *Hlf* | NM_172563 | Hepatic leukemia factor |
| *Htr7* | NM_008315 | 5-hydroxytryptamine (serotonin) receptor 7 |
| *Irf1* | NM_008390 | Interferon regulatory factor 1 |
| *Kcnma1* | NM_010610 | Potassium large conductance calcium-activated channel, subfamily M, alpha member 1 |
| *Mapk1* | NM_011949 | Mitogen-activated protein kinase 1 |
| *Mapk14* | NM_011951 | Mitogen-activated protein kinase 14 |
| *Mapk3* | NM_011952 | Mitogen-activated protein kinase 3 |
| *Mat2a* | NM_145569 | Methionine adenosyltransferase II, alpha |
| *Mtnr1a* | NM_008639 | Melatonin receptor 1A |
| *Mtnr1b* | NM_145712 | Melatonin receptor 1B |
| *Myod1* | NM_010866 | Myogenic differentiation 1 |
| *Ncoa3* | NM_008679 | Nuclear receptor coactivator 3 |
| *Nfil3* | NM_017373 | Nuclear factor, interleukin 3, regulated |
| *Nkx2-5* | NM_008700 | NK2 transcription factor related, locus 5 (Drosophila) |
| *Nms* | NM_001011684 | Neuromedin S |
| *Npas2* | NM_008719 | Neuronal PAS domain protein 2 |
| *Nr1d1* | NM_145434 | Nuclear receptor subfamily 1, group D, member 1 |
| *Nr1d2* | NM_011584 | Nuclear receptor subfamily 1, group D, member 2 |
| *Nr2f6* | NM_010150 | Nuclear receptor subfamily 2, group F, member 6 |
| *Opn3* | NM_010098 | Opsin 3 |
| *Opn4* | NM_013887 | Opsin 4 (melanopsin) |
| *Pax4* | NM_011038 | Paired box gene 4 |
| *Per1* | NM_011065 | Period homolog 1 (Drosophila) |
| *Per2* | NM_011066 | Period homolog 2 (Drosophila) |
| *Per3* | NM_011067 | Period homolog 3 (Drosophila) |
| *Pou2f1* | NM_198934 | POU domain, class 2, transcription factor 1 |
| *Ppara* | NM_011144 | Peroxisome proliferator activated receptor alpha |
| *Ppargc1a* | NM_008904 | Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha |
| *Prf1* | NM_011073 | Perforin 1 (pore forming protein) |

Supplementary Table 1. Continued

| Gene | Accession number | Description |
|---|---|---|
| *Prkaca* | NM_008854 | Protein kinase, cAMP dependent, catalytic, alpha |
| *Prkacb* | NM_011100 | Protein kinase, cAMP dependent, catalytic, beta |
| *Prkar1a* | NM_021880 | Protein kinase, cAMP dependent regulatory, type I, alpha |
| *Prkar1b* | NM_008923 | Protein kinase, cAMP dependent regulatory, type I beta |
| *Prkar2a* | NM_008924 | Protein kinase, cAMP dependent regulatory, type II alpha |
| *Prkar2b* | NM_011158 | Protein kinase, cAMP dependent regulatory, type II beta |
| *Prkca* | NM_011101 | Protein kinase C, alpha |
| *Prkcb* | NM_008855 | Protein kinase C, beta |
| *Prokr2* | NM_144944 | Prokineticin receptor 2 |
| *Ptgds* | NM_008963 | Prostaglandin D2 synthase (brain) |
| *Rora* | NM_013646 | RAR-related orphan receptor alpha |
| *Rorb* | NM_146095 | RAR-related orphan receptor beta |
| *Rorc* | NM_011281 | RAR-related orphan receptor gamma |
| *Slc9a3* | NM_001081060 | Solute carrier family 9 (sodium/hydrogen exchanger), member 3 |
| *Smad4* | NM_008540 | MAD homolog 4 (Drosophila) |
| *Sp1* | NM_013672 | Trans-acting transcription factor 1 |
| *Srebf1* | NM_011480 | Sterol regulatory element binding transcription factor 1 |
| *Stat5a* | NM_011488 | Signal transducer and activator of transcription 5A |
| *Tfap2a* | NM_011547 | Transcription factor AP-2, alpha |
| *Tef* | NM_017376 | Thyrotroph embryonic factor |
| *Tgfb1* | NM_011577 | Transforming growth factor, beta 1 |
| *Timeless* | NM_011589 | Timeless homolog (Drosophila) |
| *Wee1* | NM_009516 | WEE 1 homolog 1 (S. pombe) |
| Housekeeping genes | | |
| *Actb* | NM_007393 | Actin, beta |
| *B2m* | NM_009735 | Beta-2 microglobulin |
| *Gapdh* | NM_008084 | Glyceraldehyde-3-phosphate dehydrogenase |
| *Gusb* | NM_010368 | Glucuronidase, beta |
| *Hsp90ab1* | NM_008302 | Heat shock protein 90 alpha (cytosolic), class B member 1 |

Supplementary Table 2: Mouse DNA damage signaling pathway genes. PAMM-029ZF RT2 Profiler PCR Array (QIAGEN Sciences, Maryland, USA)

| Gene | Accession number | Description |
|---|---|---|
| *Abl1* | NM_009594 | C-abl oncogene 1, non-receptor tyrosine kinase |
| *Apex1* | NM_009687 | Apurinic/apyrimidinic endonuclease 1 |
| *Atm* | NM_007499 | Ataxia telangiectasia mutated homolog (human) |
| *Atr* | NM_019864 | Ataxia telangiectasia and rad3 related |
| *Atrx* | NM_009530 | Alpha thalassemia/mental retardation syndrome X-linked homolog (human) |
| *Bax* | NM_007527 | Bcl2-associated X protein |
| *Blm* | NM_007550 | Bloom syndrome, RecQ helicase-like |
| *Brca1* | NM_009764 | Breast cancer 1 |
| *Brca2* | NM_009765 | Breast cancer 2 |
| *Brip1* | NM_178309 | BRCA1 interacting protein C-terminal helicase 1 |
| *Cdc25a* | NM_007658 | Cell division cycle 25 homolog A (S. pombe) |
| *Cdc25c* | NM_009860 | Cell division cycle 25 homolog C (S. pombe) |
| *Cdkn1a* | NM_007669 | Cyclin-dependent kinase inhibitor 1A (P21) |
| *Chek1* | NM_007691 | Checkpoint kinase 1 homolog (S. pombe) |
| *Chek2* | NM_016681 | CHK2 checkpoint homolog (S. pombe) |
| *Dclre1a* | NM_018831 | DNA cross-link repair 1A, PSO2 homolog (S. cerevisiae) |
| *Ddb2* | NM_028119 | Damage specific DNA binding protein 2 |
| *Ddit3* | NM_007837 | DNA-damage inducible transcript 3 |
| *Ercc1* | NM_007948 | Excision repair cross-complementing rodent repair deficiency, complementation group 1 |
| *Ercc2* | NM_007949 | Excision repair cross-complementing rodent repair deficiency, complementation group 2 |
| *Exo1* | NM_012012 | Exonuclease 1 |
| *Fanca* | NM_016925 | Fanconi anemia, complementation group A |
| *Fancc* | NM_007985 | Fanconi anemia, complementation group C |
| *Fancd2* | NM_001033244 | Fanconi anemia, complementation group D2 |
| *Fancg* | NM_053081 | Fanconi anemia, complementation group G |
| *Fen1* | NM_007999 | Flap structure specific endonuclease 1 |
| *Gadd45a* | NM_007836 | Growth arrest and DNA-damage-inducible 45 alpha |
| *Gadd45g* | NM_011817 | Growth arrest and DNA-damage-inducible 45 gamma |
| *H2afx* | NM_010436 | H2A histone family, member X |
| *Hus1* | NM_008316 | Hus1 homolog (S. pombe) |
| *Lig1* | NM_010715 | Ligase I, DNA, ATP-dependent |

Supplementary Table 2. Continued

| Gene | Accession number | Description |
|---|---|---|
| *Mbd4* | NM_010774 | Methyl-CpG binding domain protein 4 |
| *Mcph1* | NM_173189 | Microcephaly, primary autosomal recessive 1 |
| *Mdc1* | NM_001010833 | Mediator of DNA damage checkpoint 1 |
| *Mgmt* | NM_008598 | O-6-methylguanine-DNA methyltransferase |
| *Mif* | NM_010798 | Macrophage migration inhibitory factor |
| *Mlh1* | NM_026810 | MutL homolog 1 (E. coli) |
| *Mlh3* | NM_175337 | MutL homolog 3 (E coli) |
| *Mpg* | NM_010822 | N-methylpurine-DNA glycosylase |
| *Mre11a* | NM_018736 | Meiotic recombination 11 homolog A (S. cerevisiae) |
| *Msh2* | NM_008628 | MutS homolog 2 (E. coli) |
| *Msh3* | NM_010829 | MutS homolog 3 (E. coli) |
| *Nbn* | NM_013752 | Nibrin |
| *Nthl1* | NM_008743 | Nth (endonuclease III)-like 1 (E.coli) |
| *Ogg1* | NM_010957 | 8-oxoguanine DNA-glycosylase 1 |
| *Parp1* | NM_007415 | Poly (ADP-ribose) polymerase family, member 1 |
| *Parp2* | NM_009632 | Poly (ADP-ribose) polymerase family, member 2 |
| *Pcna* | NM_011045 | Proliferating cell nuclear antigen |
| *Pms2* | NM_008886 | Postmeiotic segregation increased 2 (S. cerevisiae) |
| *Pole* | NM_011132 | Polymerase (DNA directed), epsilon |
| *Polh* | NM_030715 | Polymerase (DNA directed), eta (RAD 30 related) |
| *Poli* | NM_011972 | Polymerase (DNA directed), iota |
| *Ppm1d* | NM_016910 | Protein phosphatase 1D magnesium-dependent, delta isoform |
| *Ppp1r15a* | NM_008654 | Protein phosphatase 1, regulatory (inhibitor) subunit 15A |
| *Prkdc* | NM_011159 | Protein kinase, DNA activated, catalytic polypeptide |
| *Pttg1* | NM_013917 | Pituitary tumor-transforming gene 1 |
| *Rad1* | NM_011232 | RAD1 homolog (S. pombe) |
| *Rad17* | NM_011233 | RAD17 homolog (S. pombe) |
| *Rad18* | NM_021385 | RAD18 homolog (S. cerevisiae) |
| *Rad21* | NM_009009 | RAD21 homolog (S. pombe) |
| *Rad50* | NM_009012 | RAD50 homolog (S. cerevisiae) |
| *Rad51* | NM_011234 | RAD51 homolog (S. cerevisiae) |

Supplementary Table 2. Continued

| Gene | Accession number | Description |
|---|---|---|
| *Rad51c* | NM_053269 | Rad51 homolog c (S. cerevisiae) |
| *Rad51b* | NM_009014 | RAD51-like 1 (S. cerevisiae) |
| *Rad52* | NM_011236 | RAD52 homolog (S. cerevisiae) |
| *Rad9a* | NM_011237 | RAD9 homolog (S. pombe) |
| *Rev1* | NM_019570 | REV1 homolog (S. cerevisiae) |
| *Rnf8* | NM_021419 | Ring finger protein 8 |
| *Rpa1* | NM_026653 | Replication protein A1 |
| *Smc1a* | NM_019710 | Structural maintenance of chromosomes 1A |
| *Smc3* | NM_007790 | Structural maintenace of chromosomes 3 |
| *Sumo1* | NM_009460 | SMT3 suppressor of mif two 3 homolog 1 (yeast) |
| *Terf1* | NM_009352 | Telomeric repeat binding factor 1 |
| *Topbp1* | NM_176979 | Topoisomerase (DNA) II binding protein 1 |
| *Trp53* | NM_011640 | Transformation related protein 53 |
| *Trp53bp1* | NM_013735 | Transformation related protein 53 binding protein 1 |
| *Ung* | NM_011677 | Uracil DNA glycosylase |
| *Wrn* | NM_011721 | Werner syndrome homolog (human) |
| *Xpa* | NM_011728 | Xeroderma pigmentosum, complementation group A |
| *Xpc* | NM_009531 | Xeroderma pigmentosum, complementation group C |
| *Xrcc1* | NM_009532 | X-ray repair complementing defective repair in Chinese hamster cells 1 |
| *Xrcc2* | NM_020570 | X-ray repair complementing defective repair in Chinese hamster cells 2 |
| *Xrcc3* | NM_028875 | X-ray repair complementing defective repair in Chinese hamster cells 3 |
| *Xrcc6* | NM_010247 | X-ray repair complementing defective repair in Chinese hamster cells 6 |
| Housekeeping genes | | |
| *Actb* | NM_007393 | Actin, beta |
| *B2m* | NM_009735 | Beta-2 microglobulin |
| *Gapdh* | NM_008084 | Glyceraldehyde-3-phosphate dehydrogenase |
| *Gusb* | NM_010368 | Glucuronidase, beta |
| *Hsp90ab1* | NM_008302 | Heat shock protein 90 alpha (cytosolic), class B member 1 |

# Chapter 4.

# Do 50/60 Hz magnetic fields influence oxidative or DNA damage responses in human SH-SY5Y neuroblastoma cells?

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ORIGINAL ARTICLE



# Do 50/60 Hz magnetic fields influence oxidative or DNA damage responses in human SH-SY5Y neuroblastoma cells?

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**ABSTRACT**

**Purpose:** We investigated the possible effects of 50 and 60 Hz magnetic fields (MFs) on reactive oxygen species (ROS) production, DNA damage, DNA damage repair rate, as well as gene expression related to oxidative stress and DNA damage signaling.

**Materials and methods:** Human SH-SY5Y neuroblastoma cells were sham-exposed or exposed to 100 $\mu T_{RMS}$ MFs for 24 h, then assayed or further treated with 100 μM menadione for 1 h before the assay. The levels of ROS and cytosolic superoxide anion ($O_2^{\bullet -}$) were assayed fluorometrically. DNA damage and gene expression were assayed by comet assay and RT-qPCR, respectively. To examine whether MFs affected DNA damage repair rate, cells were allowed to repair their DNA for 1 or 2 h after menadione treatment and then assayed for DNA damage.

**Results:** There was suggestive evidence of a general low-magnitude increase in the expression of ROS-related genes (primarily genes with antioxidant activity) when quantified immediately after MF exposure, suggesting a response to a small increase in ROS level. The possible upregulation of ROS-related genes is supported by the finding that the level of menadione-induced ROS was consistently decreased by 50 Hz MFs (not significantly by 60 Hz MFs) in several measurements 30–60 min after MF exposure. MF exposures did not affect cytosolic $O_2^{\bullet -}$ levels, DNA damage, or its repair rate. Changes in the expression of DNA damage-signaling genes in the MF-exposed cells did not exceed the expected rate of false-positive findings. No firm evidence was found for differential effects from 50 *vs.* 60 Hz MFs.

**Conclusions:** While only weak effects were found on the endpoints measured, the results are consistent with MF effects on ROS signaling.

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Extremely low-frequency magnetic fields; reactive oxygen species (ROS); DNA damage; DNA repair

## Introduction

Humans are ubiquitously exposed to environmental extremely low-frequency (ELF) magnetic fields (MFs) from electricity use and transmission. Over the last decades, questions have been raised about the health consequences of exposure to these fields. Epidemiological studies have rather consistently reported an increased risk of childhood leukemia associated with residential exposure to weak MFs with magnetic flux density above 0.3–0.4 μT from power lines (Ahlbom et al. 2000). These epidemiological findings were the main reason that prompted the International Agency for Research on Cancer (IARC) to classify ELF MFs as possibly carcinogenic to humans (IARC 2002). However, in vitro and animal studies provided inadequate evidence for carcinogenic effects. In addition, after almost two decades since the IARC classification, there is still no generally accepted mechanism shown to underlie the possible association between exposure to ELF MFs and cancer. Among the most plausible primary mechanisms is the so-called radical pair mechanism (Hore and Mouritsen 2016). This mechanism provides a physicochemical link between MFs and radical reactions, which have many essential roles in biology. Much of the discussion on the role of radicals in ELF MF effects focus on reactive oxygen species (ROS) and oxidative stress (the imbalance between oxidants and antioxidants in favor of the former).

As ROS can damage macromolecules, including DNA, MF-induced oxidative stress offers a seemingly simple explanation for the possible carcinogenic effects of ELF MFs. There is, however, a major problem with this explanation: the expected magnitude (as predicted by the radical pair mechanism) of the effect of environmental ELF MFs on the radical levels is small (Hore 2019), and it is hard to see how weak environmental fields could cause biologically meaningful changes in radical levels in the presence of the relatively strong geomagnetic field (Juutilainen et al. 2018). Therefore, if ELF MFs cause detectable effects on cellular ROS levels,

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Supplemental data for this article can be accessed here.

such effects are likely to be secondary changes rather than a direct consequence of altered radical yield through the radical pair mechanism. A plausible sequence of events might include detecting the MF by biological molecules involving radical pairs and consequent changes in cellular signaling (Juutilainen et al. 2018). ROS have multiple roles in cell signaling, including DNA damage signaling (Shackelford et al. 2000; Sancar et al. 2004; Wang et al. 2016; Mori et al. 2017). The size of MF effect on ROS has generally been small in our previous studies (Luukkonen et al. 2014; Kesari et al. 2015, 2016; Höytö et al. 2017) and other studies reporting ELF MF effects on ROS (Mattsson and Simkó 2014; Juutilainen et al. 2018), suggesting MF effects on ROS signaling rather than induction of oxidative stress. However, these studies have not directly addressed ELF MF effects on the ROS signaling pathway.

In this study, we investigated whether ELF MFs could affect the ROS signaling pathway by assaying the expression of genes related to ROS formation, ROS metabolism, and defense against oxidative stress. We also examined the possible effects of ELF MFs on the levels of ROS and cytosolic superoxide anion radical ($O_2^{\bullet-}$) and explored whether ELF MFs could induce DNA damage or influence DNA damage repair or gene expression related to DNA damage signaling. All our previous studies were conducted using 50 Hz MFs. As frequency-specific effects could potentially help to understand different responses to ELF MFs and the (static) geomagnetic field, experiments in the present study included both 50 or 60 Hz, the two power-line frequencies used in different countries around the world. In our experiments, we used human SH-SY5Y neuroblastoma cells, a widely used in vitro model in studies investigating the biological effect of MFs (Calabrò et al. 2013; Luukkonen et al. 2014; Vergallo et al. 2014; Benassi et al. 2016; Höytö et al. 2017). The flux density of the applied MFs was 100 $\mu T_{RMS}$, chosen based on previous studies in which this flux density modified biological responses in SH-SY5Y cells (Markkanen et al. 2008; Luukkonen et al. 2011, 2014, 2017; Kesari et al. 2015; Höytö et al. 2017). In addition to exploring the effects of exposure to sole MFs, we examined whether MFs can alter cellular responses to menadione (a chemical known to induce intracellular ROS production and cause DNA damage).

## Materials and methods

### Cell culture

We conducted the experiments using human SH-SY5Y neuroblastoma cells (obtained from Dr. Sven Påhlman, University of Uppsala, Sweden). Cells were grown in Dulbecco's modified Eagle medium containing 4.5 g/l glucose (Gibco, Paisley, UK) and supplemented with 10% v/v heat-inactivated fetal bovine serum, 50 U/ml penicillin, and 50 µg/ml streptomycin. Cell cultures were maintained at a density between 1 and $2.5 \times 10^5$ cells/cm$^2$ in 75 cm$^2$ EasYFlask™ cell culture flasks (Nunclon, Roskilde, Denmark) and kept in a humidified atmosphere containing 5% $CO_2$ at 37 °C in a Panasonic MCO-170AICUV cell culture incubator (Panasonic Healthcare Co., Japan). Cells were subcultured every 3–4 days and harvested with 0.02% w/v Ethylenediaminetetraacetic acid (EDTA) in phosphate buffer saline (PBS; 2.7 mM KCl, 1.5 mM $KH_2PO_4$, 137 mM NaCl, 8.1 mM $Na_2HPO_4$), w/o $Ca^{+2}$ and $Mg^{+2}$. The count and viability of the cells were determined with LUNA-II™ automated cell counter (Logos Biosystems, Gyeonggi-do, South Korea) after staining with 0.4% trypan blue.

For the experiments, cells were cultured 24 h before the exposure. In gene expression and comet assay experiments, a count of $2 \times 10^6$ cells was seeded in 5 ml media per dish ($60 \times 15$ mm Nunclon™ Delta cell culture dish, Nunclon, Roskilde, Denmark). For assaying the levels of ROS and cytosolic $O_2^{\bullet-}$, a count of $1 \times 10^5$ cells was seeded in 0.5 ml medium per well in Costar® 48-well plates (Corning, NY, USA).

### Exposure of the cells

A comprehensive description of the exposure system and its integrated computerized blinding system was previously reported in our earlier paper (Mustafa et al. 2021). Briefly, an identical pair of three square copper coil systems in a Merritt-like configuration generated horizontal MFs. These two coils systems were housed in two identical Panasonic MCO-170AICUV cell culture incubators where the temperature and atmosphere were monitored and autoregulated (+37 °C, 5% $CO_2$).

Sinusoidal MF signal (at 50 or 60 Hz) was generated and amplified by BK4052 5 MHz Waveform Generator (B&K Precision, California, USA) and EP4000 Power Amplifier (Behringer, Willich, Germany), respectively. For cell exposure, the magnetic flux density was set to 100 $\mu T_{RMS}$ and monitored with TM-192 triaxial MF meter (Tenmars, Taipei, Taiwan). The flux density of the background low-frequency MFs in the incubators was $<2$ $\mu T_{RMS}$. The maximum static geomagnetic field in the incubators was perpendicular to the applied MFs, and its intensity (~30 µT) was measured with GM08 Gaussmeter (Hirst, Cornwall, UK). The culture vessels were placed in the center of the coil systems to ensure a uniform MF exposure and identical condition for sham exposure. Field homogeneity in the position of culture vessels was >98%. The temperature was monitored with a 52 K/J digital thermometer (Fluke, Washington, USA), and no difference between the exposure incubators was observed during the experiments. No mechanical vibration was detected by B&K 4366 accelerometer (Bruel & Kjaer, Naerum, Denmark). In every new experiment, the computerized blinding system randomly selected one of the two coil systems to apply the current. The other coil system served as a sham exposure unit with no current flowing in the coils. The blinding system random selections were automatically recorded to be revealed after sample analyses.

### Experimental protocol

A flowchart of the experimental procedures is presented in Figure 1. Cells were exposed to sham exposure or 50/60 Hz MFs for 24 h. For assaying the expression of 84 oxidative

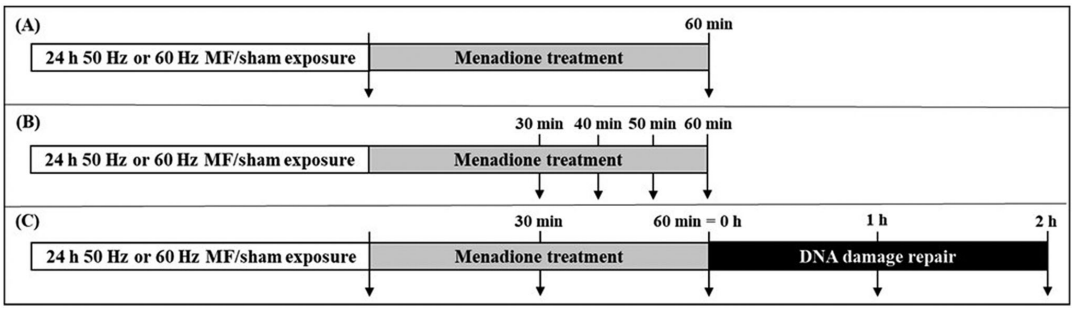


Figure 1. Experimental procedures flowchart. The arrows (↓) indicate the time points at which samples were collected to assay (A) gene expression, (B) ROS and cytosolic $O_2^{\bullet-}$ levels, and (C) levels of DNA damage and its repair rate; the end of incubation period with or without menadione ('60 min') coincides with the '0 h' of DNA damage repair.

stress-related genes (QIAGEN PAHS-065YA RT$^2$ Profiler PCR Array; Supplementary Table 1) and 84 DNA damage-signaling involved genes (QIAGEN PAHS-029ZA RT$^2$ Profiler PCR Array; Supplementary Table 2), cells were collected after exposure for reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR) or further treated with 100 µM menadione for 1 h, then collected.

The fluorometric probes 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Invitrogen, California, USA) and Dihydroethidium (DHE; Sigma, Buchs, Switzerland) were used to assay the production of ROS and cytosolic $O_2^{\bullet-}$, respectively. In these experiments, the exposed cells were treated with menadione for 30, 40, 50 min, or 1 h, then assayed. According to the fluorescent probe manufacturers, cells should be incubated with the probes for at least 30 min before the measurement. Thus, loading the cells with the probes and the chemical treatment started simultaneously.

To explore the effect of MFs on DNA damage level, the exposed cells were assayed immediately via comet assay or further incubated in menadione-free or menadione-containing media for 30 min or 1 h, then assayed. For investigating whether MFs modify the repair rate of DNA damage induced by menadione, cells were incubated in fresh media for 1 or 2 h to allow for DNA repair after chemical treatment, then assayed for DNA damage.

The menadione treatment parameters used in the present study were selected based on preliminary experiments and previous studies investigating co-exposure effects using menadione as DNA damaging/ROS-inducing agent (Luukkonen et al. 2011, 2017; Kesari et al. 2015, 2016).

### RT-qPCR

Cells were collected by scraping in PBS w/o $Ca^{+2}$ or $Mg^{+2}$, centrifuged, snap-frozen in liquid nitrogen, and stored at −80 °C for the later RNA extraction. As in our earlier study (Mustafa et al. 2021), RNA was extracted using RNeasy® Mini Kit (QIAGEN, Hilden, Germany) and assayed for purity and quantity with NanoDrop® ND-1000 ultraviolet-visible spectrophotometer (ThermoFisher Scientific, Waltham, USA). The extracted RNA was reverse transcripted into Complementary DNA (cDNA) using RT$^2$ First Strand Kit (QIAGEN, Maryland, USA). The synthesized cDNA was then used as a template for SYBR-Green I fluorescence-based qPCR reactions. The reactions were run as instructed by RT$^2$ Profiler™ PCR Array Handbook in LightCycler® 480 II (Roche, Basel, Switzerland). At the end of each run, LightCycler® software determined a quantification cycle ($C_q$) value for every assayed gene. These values were normalized to the average expression of five reference genes: *ACTB*, *B2M*, *GAPDH*, *HPRT1*, and *RPLP0*. The $2^{-\Delta\Delta Cq}$ method (as originally described by Livak and Schmittgen 2001) was used to calculate gene expression fold changes (MF/sham).

### Fluorometric assay of ROS and cytosolic $O_2^{\bullet-}$

After sham or MF exposure, cells were washed and then loaded with the fluorescent probes in the absence or the presence of menadione. DCFH-DA (40 µM) was used as a fluorescent probe to measure ROS level at 485/535 nm excitation/emission wavelengths. DHE (10 µM) was used as a fluorescent probe to measure the levels of cytosolic $O_2^{\bullet-}$ at 492/595 nm. Of notice, DCFH-DA is poor at detecting $O_2^{\bullet-}$ (LeBel et al. 1992); thus, DHE was used as a specific probe for cytosolic $O_2^{\bullet-}$. The fluorometric measurements were done with Infinite® 200 PRO microplate reader (Tecan, Männedorf, Switzerland). The measured fluorescence intensity was expressed in relative fluorescence units (RFUs) by Magellan™ data analysis software (Tecan, Männedorf, Switzerland).

### Comet assay

Comet assay or single cell gel electrophoresis was performed to evaluate DNA damage and its repair rate. A detailed description of the procedures and reagents used in the assay was previously reported in our earlier paper (Mustafa et al. 2021). Briefly, cells were scrapped, centrifuged, and resuspended in PBS w/o $Ca^{+2}$ and $Mg^{+2}$ at a final concentration of $1 \times 10^6$ cells/ml. From each sample suspension, 15 µl ($\sim 1.5 \times 10^4$ cells) were mixed with 75 µl 0.5% low melting point agarose. These mixtures were spread using coverslips on coded microscope slides precoated with a thin layer of 1% normal melting point agarose and left on ice for at least 5 min to solidify. After that, coverslips were removed, and

slides were immersed in Triton x-100 lysis buffer for 1 h at 4 °C in dark conditions. Thereafter, DNA was allowed to unwind by placing the slides in the NaOH-based electrophoresis buffer at room temperature for 15 min. The electrophoresis was run at 380 mA and 24 V (0.6 V/cm) for another 15 min using Horizon 20.25 Electrophoresis System (Life Technologies, Baltimore, USA). The electrophoresis occurred in an alkaline condition (pH > 13) to assay DNA single- and double-strand breaks (Singh and Lai 1998). Subsequently, slides were neutralized in trisaminomethane buffer (pH 7.5, 3 × 5 min) and then fixed by immersion in 96% ethanol for 1 min. For blinded analysis of the coded slides, they were stained with 20 µg/ml ethidium bromide fluorescent dye. AxioImager A1 microscope (Carl Zeiss, Göttingen, Germany) was used. The microscope was connected to Comet assay IV image analysis software (Perceptive Instruments, Haverhill, UK). Olive tail moment (OTM), a measure of tail length × a measure of DNA in the tail, was used to indicate the extent of DNA damage.

### Statistical analyses

As earlier (Mustafa et al. 2021), data were analyzed in IBM® SPSS® data analysis software using two-way and three-way ANOVA. The fixed factors were MF exposure, menadione treatment duration, and DNA damage repair duration. The dependent variables were normalized Cq values, RFUs, and normalized OTMs. The replicate effect was considered a random factor. Data were from 3 to 4 independent experiments. $p$-Value <.05 was considered statistically significant. In gene expression analyses, multiple testing correction of the $p$-values was not used because the purpose of our study was exploratory and hypothesis-generating (Streiner and Norman 2011), aiming at identifying potential MF-responsive genes for further studies.

## Results

### Oxidative stress-related genes

Clear responses to menadione were observed (Figure 2, Supplementary Table 3). Four of the oxidative stress-related genes studied showed either a statistically significant up-regulation (*DUSP1*, *HSPA1A*, *SQSTM1*) or down-regulation (*FHL2*) to menadione in all exposure conditions (with or without MFs). Most of the genes that were statistically significantly up- or down-regulated in only some of the conditions showed small fold changes (<2.0), so the differences in statistical significance are most likely due to chance (false

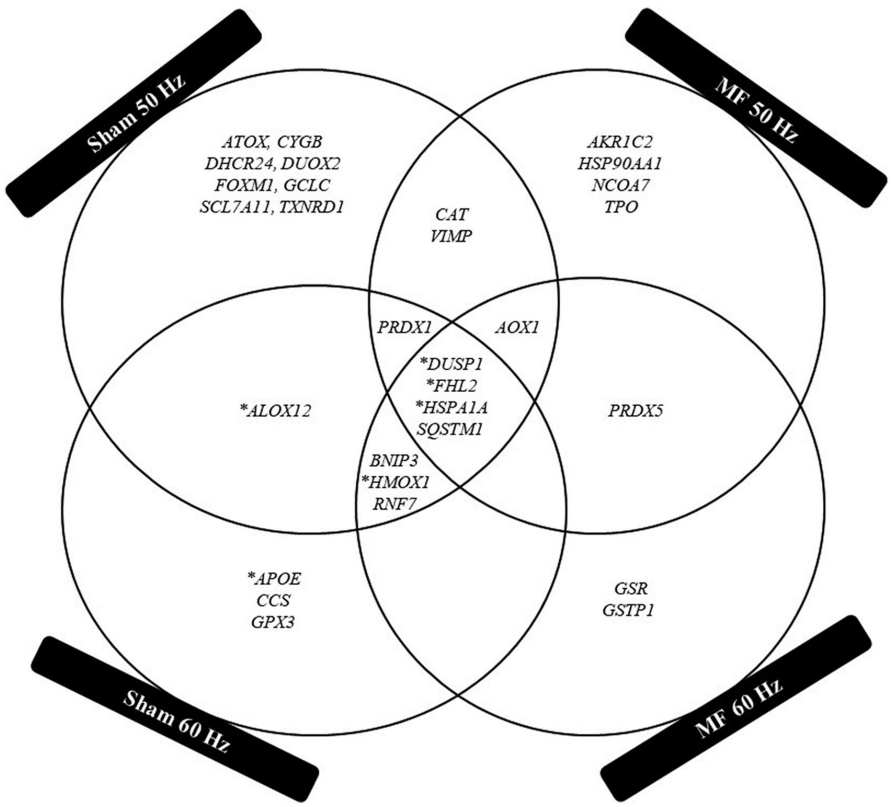


Figure 2. Oxidative stress-related genes showing statistically significant changes in response to menadione treatment with or without MF exposure. The asterisk (*) indicates changes (up- or down-regulation) of >2-fold changes.

positive or false negative findings in some of the conditions). A suggestive modification of the menadione effect by MF exposure was seen in two cases. The expression of *ALOX12* was increased in both sham exposures (fold changes with SEM: 2.09 ± 0.16 and 3.98 ± 0.71), but not significantly in the MF-exposed groups. *HMOX1* was upregulated after sham exposures (2.95 ± 0.28 and 3.606 ± 0.430) and in the cells exposed to 60 Hz MFs (3.849 ± 0.854), but not significantly in the 50 Hz MF group. However, examination of the data revealed that the fold changes were of similar direction and magnitude in all treatment groups, so the lack of significance in the MF groups was apparently due to chance.

Another way to evaluate possible MF effects on gene expression is to compare the MF-exposed groups to corresponding sham-exposed groups (Table 1). All statistically significant differences observed immediately after MF exposure (before menadione treatment) were upregulations, both for the 50 and 60 Hz MF exposures. This pattern may be worth noting, as the expected number of false positives is two upregulations and two downregulations in each experimental condition (84 genes are measured, so there will be, on the average, 4.1 'statistically significant' findings just by chance when $p = .05$ is used as the limit for significance; it can be assumed that half of these false positives will be upregulations and the other half downregulations). Only one of these upregulations (i.e. *GCLC*) was seen in both the 50 and 60 Hz MF groups. However, the fold changes were generally small; none of the increases showed a fold change >2, so the chance is a plausible explanation for false positive or false negative findings in one of the MF groups.

**Table 1.** Oxidative stress-related genes showing statistically significant changes in response to MFs. Human SH-SY5Y neuroblastoma cells were sham-exposed or exposed to 100 $\mu T_{RMS}$ MFs at 50 or 60 Hz for 24 h. After exposure, cells were either immediately assayed for gene expression (0 min) or further incubated in menadione-containing or menadione-free media for 1 h, then assayed. Fold changes (MF exposure/sham exposure ± standard error of the mean) are from three independent experiments, $n = 3$. $^{*}p < .05$, $^{**}p < .01$.

| MF | Time | Menadione | Upregulated genes | Downregulated genes |
|---|---|---|---|---|
| 50 Hz | 0 min | No | *FOXM1* 1.059 ± 0.014* | – |
| | | | *GCLC* 1.049 ± 0.010* | |
| | | | *HMOX1* 1.667 ± 0.144* | |
| | | | *HSP90AA1* 1.028 ± 0.006* | |
| | | | *PRNP* 1.974 ± 0.241* | |
| | | | *RNF7* 1.079 ± 0.015* | |
| | 1 h | No | *DUSP1* 1.028 ± 0.005* | *GPX3* 0.922 ± 0.014* |
| | | Yes | *APOE* 1.501 ± 0.038** | *CAT* 0.943 ± 0.010* |
| | | | | *DHCR24* 0.941 ± 0.012** |
| | | | | *NCOA7* 0.937 ± 0.011* |
| | | | | *SIRT2* 0.945 ± 0.011* |
| 60 Hz | 0 min | No | *ALOX12* 1.367 ± 0.089* | – |
| | | | *FHL2* 1.220 ± 0.039* | |
| | | | *GCLC* 1.117 ± 0.017* | |
| | | | *PRDX3* 1.143 ± 0.032* | |
| | | | *TXN* 1.234 ± 0.044* | |
| | | | *UCP2* 1.166 ± 0.013** | |
| | 1 h | No | – | *FOXM1* 0.932 ± 0.012* |
| | | | | *HMOX1* 0.769 ± 0.034* |
| | | Yes | – | *AOX1* 0.646 ± 0.030* |
| | | | | *BAG2* 0.863 ± 0.022* |
| | | | | *CCS* 0.859 ± 0.030* |
| | | | | *NCF1* 0.743 ± 0.049* |

Two gene expression differences showed a fold change >1.5 in the 50 Hz MF group (*HMOX1* 1.667 ± 0.144, *PRNP* 1.974 ± 0.241) (Table 1). Although the expression of *HMOX1* was not statistically significantly altered in the 60 Hz MF group, the direction of the fold change was similar (1.212 ± 0.528). *PRNP* showed a potentially different response to 50 *vs.* 60 Hz MFs; it was significantly upregulated in the 50 Hz MF group but showed a fold change value close to 1.0 in the 60 Hz group (0.959 ± 0.308).

The statistically significant differences observed at 1 h after MF exposure (with or without menadione exposure) were generally small (fold changes <1.5), and the number of statistically significant differences did not clearly differ from the expected number of chance findings.

### DNA damage signaling-related genes

Clear responses to menadione were observed (Figure 3, Supplementary Table 4). Seven of the DNA damage signaling-related genes studied showed either a statistically significant up-regulation (*DDIT3*, *GADD45G*, *H2AFX*, *PPM1D*, *PPP1R15A*, *RNF8*) or down-regulation (*SMC1A*) to menadione in all exposure conditions (with or without MFs). Most of the genes that were statistically significantly up-or down-regulated in only some of the conditions showed small fold changes (<2.0), so the differences in statistical significance are most likely due to chance.

Comparison of the MF-exposed groups to the corresponding sham-exposed groups (with or without menadione) showed only small fold changes and no marked deviations from the expected number of false-positive findings (Table 2), indicating that the MFs used did not affect DNA damage signaling or alter the responses to menadione.

### ROS and cytosolic $O_2^{\bullet-}$

Compared to sham exposure, MFs reduced the levels of menadione-induced ROS over all the assayed time points (Figure 4(A,B)). This overall effect was statistically significant in the case of 50 Hz exposure ($p = .037$, Figure 4(A)) but not significant in the case of 60 Hz exposure ($p = .165$, Figure 4(B)). Concerning individual time points, 50 Hz MFs significantly reduced the levels of menadione-induced ROS after menadione treatment durations of 40 min (13% reduction, $p = .036$), 50 min (12% reduction, $p = .034$), and 1 h (11% reduction, $p = .036$). The reduction after the 30-min menadione treatment (14%) was not statistically significant ($p = .052$, Figure 4(A)).

No effects on menadione-induced cytosolic $O_2^{\bullet-}$ levels were observed; the differences between MF-exposed and sham-exposed cells were not statistically significant, and the direction of the differences was not consistent (Figure 4(C,D)). Moreover, exposure to sole 50 or 60 Hz MFs did not affect ROS or cytosolic $O_2^{\bullet-}$ levels (Figure 4(A–D)).

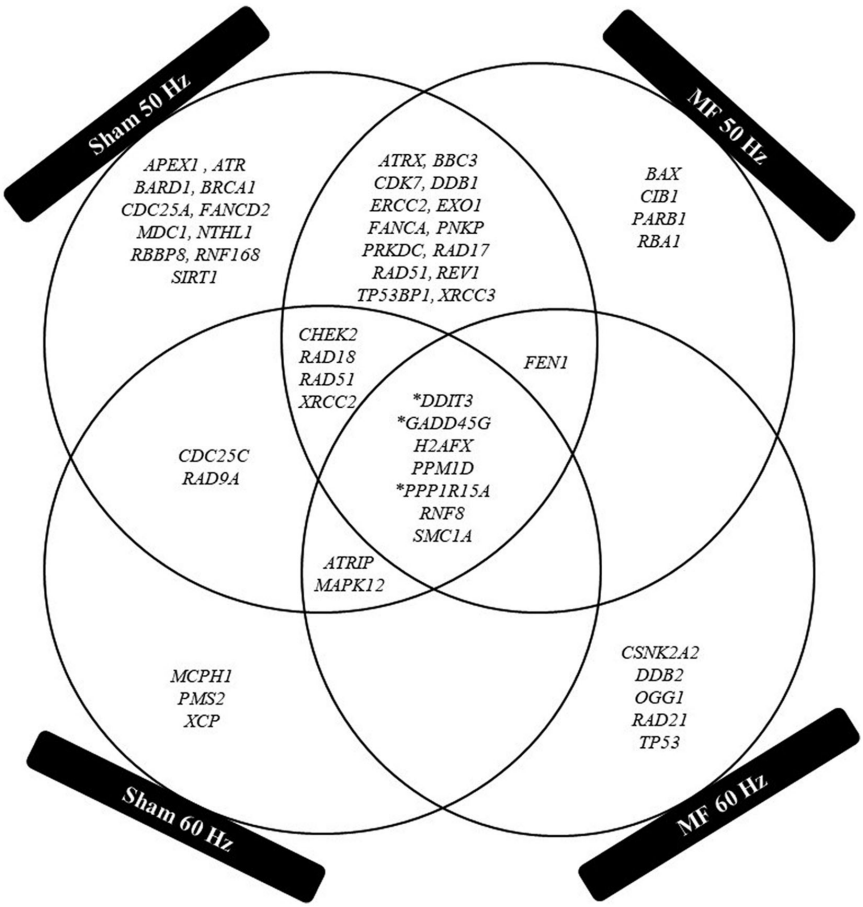


**Figure 3.** DNA damage signaling-related genes showing statistically significant changes in response to menadione treatment with or without MF exposure. The asterisk (*) indicates changes (up- or down-regulation) of >2-fold changes.

**Table 2.** DNA damage signaling-related genes showing statistically significant changes in response to MFs. Human SH-SY5Y neuroblastoma cells were sham-exposed or exposed to 100 $\mu T_{RMS}$ MFs at 50 or 60 Hz for 24 h. After exposure, cells were either immediately assayed for gene expression (0 min) or further incubated in menadione-containing or menadione-free media for 1 h, then assayed. Fold changes (MF exposure/sham exposure ± standard error of the mean) are from three independent experiments, $n = 3$. *$p < .05$, **$p < .01$.

| MF | Time | Menadione | Upregulated genes | Downregulated genes |
|---|---|---|---|---|
| 50 Hz | 0 min | No | – | – |
| | 1 h | No | *BBC3* 1.130 ± 0.024* | *EXO1* 0.966 ± 0.006* |
| | | | *CDC25A* 1.155 ± 0.036* | *OGG1* 0.916 ± 0.017* |
| | | | | *TP73* 0.926 ± 0.013* |
| | | Yes | *GADD45A* 1.158 ± 0.038* | – |
| | | | *TOPBP1* 1.084 ± 0.013* | |
| 60 Hz | 0 min | No | *FANCG* 1.091 ± 0.019* | – |
| | | | *MRE11A* 1.037 ± 0.008* | |
| | 1 h | No | *PNKP* 1.077 ± 0.004** | *SMC1A* 0.904 ± 0.019* |
| | | | *PPP1R15A* 1.077 ± 0.011* | |
| | | Yes | – | *CRY1* 0.894 ± 0.022* |
| | | | | *GADD45G* 0.881 ± 0.003** |
| | | | | *PMS1* 0.876 ± 0.018* |
| | | | | *TOPBP1* 0.945 ± 0.007* |

### *DNA damage and its repair rate*

Compared to sham exposure, MFs alone (50 or 60 Hz) did not change DNA damage levels assayed immediately after the exposure; neither did they significantly modify the DNA damage induced by 30-min or 1-h menadione treatments (Figure 5(A,B)). In cells exposed to 50 Hz MFs, the OTM value was slightly higher in the MF + Menadione group than in the Sham + Menadione group after both 30-min and 1-h menadione treatments (Figure 5(A)); however, this difference was not statistically significant ($p = .126$). Moreover, MF exposure did not affect the repair rate of the DNA damage induced by 1-h menadione treatment assessed immediately after the treatment ('0 min'), or 1 or 2 h after it (Figure 5(C,D); also see Figure 1(C)).

## Discussion

Clear responses to menadione were seen in all endpoints measured, indicating that the assays worked as expected.

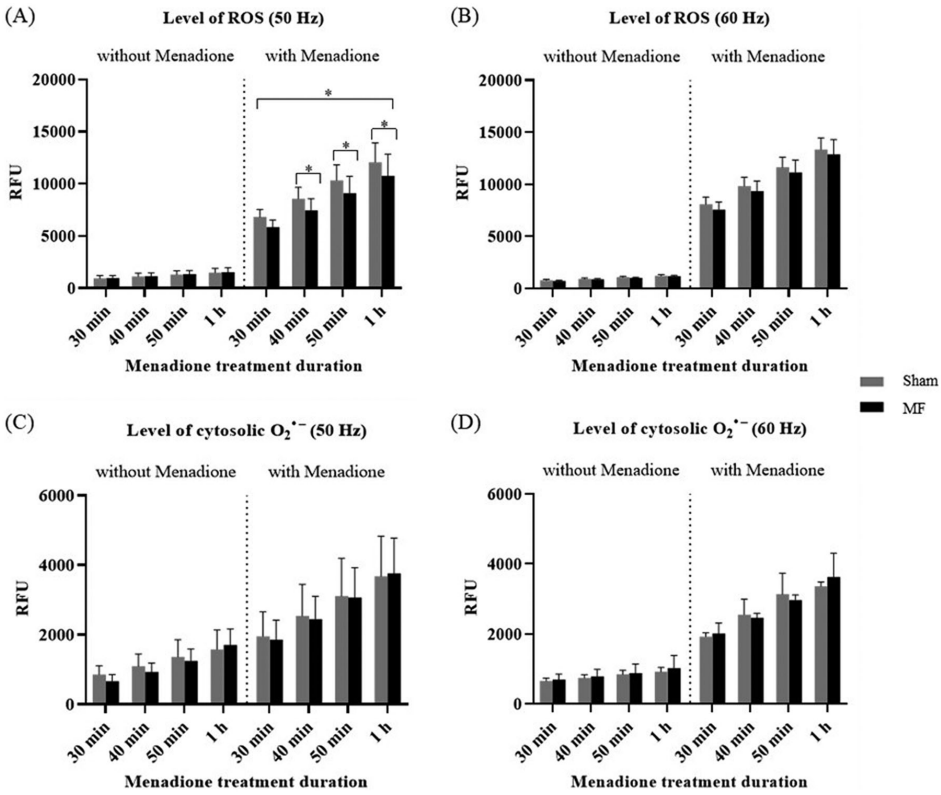


Figure 4. Levels of ROS (A,B) and cytosolic $O_2^{\bullet-}$ (C,D) measured 30, 40, 50 min, or 1 h after sham exposure or 100 $\mu T_{RMS}$ MF exposure at 50 or 60 Hz for 24 h, with or without menadione treatment. Relative fluorescence units (RFU) are the means of fluorometric measurements. Error bars represent the standard errors of the means. $n = 3–4$. $^*p < .05$.

The most obvious MF effect was the reduction of menadione-induced ROS levels in cells exposed to 50 Hz fields, observed systematically with all menadione treatment durations.

No obvious candidates for MF-responsive genes were identified in the gene expression experiments. However, several oxidative stress-related genes were found to be upregulated when quantified immediately at the end of MF exposure and before any menadione treatment. The upregulated genes were not consistently the same in the 50 and 60 Hz experiments—only *GCLC* was statistically significantly increased by both 50 and 60 Hz exposures, but its fold changes (1.049, 1.117) were very close to 1.0. As the number of statistically significant upregulations was higher than the expected number of false-positive findings in both 50 and 60 Hz experiments, these upregulations may nevertheless be worth noting. All the upregulated genes are found to be involved in responses to oxidative stress (Gelain et al. 2009; Raghunath et al. 2018). A possible interpretation of this upregulation is that the MF exposure caused a small increase in ROS level, resulting in a general low-magnitude increase in the expression of several oxidative stress-inducible genes. In this interpretation, the differences between 50 and 60 Hz MFs may not be meaningful, as chance may determine which low-magnitude upregulations become statistically significant in each experiment. With respect to the change in the expression of individual genes, our findings on *HMOX1* and *ALOX12* are in line with previous studies reporting that MF exposure increased the expression of these genes (Akbarnejad et al. 2017; Costantini et al. 2019; Zhou et al. 2019). It is also worth noting that our results show a lack of effects on *CRY1* (the gene that encodes cryptochrome circadian regulator-1 protein). The single significant but small deviation from 1.0 in *CRY1* expression observed 1 h after 60 Hz exposure (Table 2) is most likely a chance finding. Cryptochromes are magnetosensitive flavoproteins, and are of particular interest, as several research groups have suggested that cryptochromes are key molecules in responses to MFs (Lagroye et al. 2011; Vanderstraeten et al. 2012; Juutilainen et al. 2018). The null findings of the present study on *CRY* expression support the results from our previous study (Mustafa et al. 2021) and those from Lundberg et al. (2019) but are inconsistent with the findings from Manzella et al. (2015). This discrepancy may be related to differences in the biological models and experimental protocols used. Anyway, lack of effect on *CRY* expression in the present study shows that altered *CRY* expression (measured immediately after MF exposure) is not required to reduce

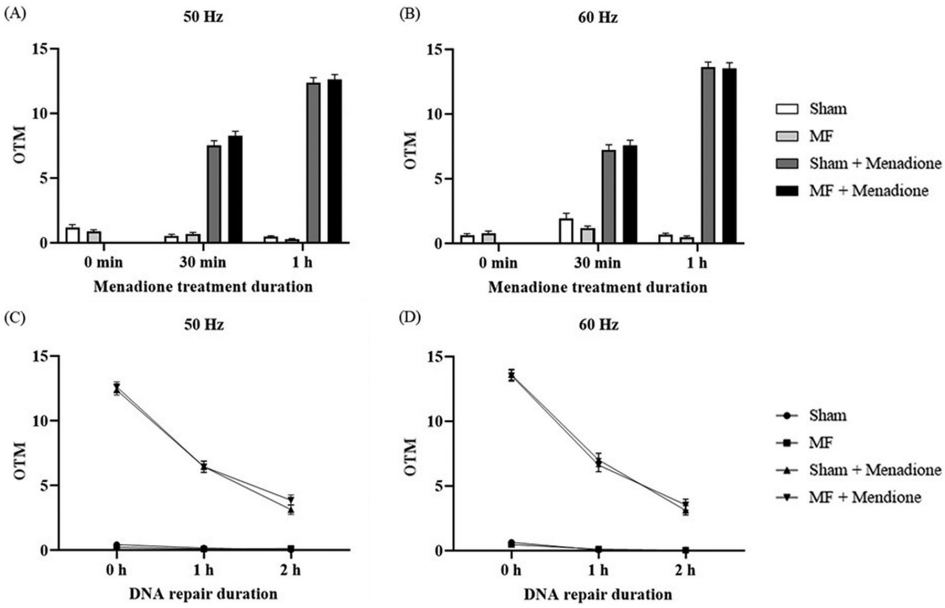


**Figure 5.** Effects of 50 and 60 Hz MFs on DNA damage (A,B, respectively) and DNA damage repair rate (C,D, respectively). (A,B) DNA damage levels were assayed immediately after sham/MF exposure ('0 min'), or 30 min or 1 h after the exposure with or without menadione treatment. (C,D) DNA damage repair rate assayed 1 h after the exposure, with or without menadione treatment (i.e. 0 h of DNA repair duration), and then again 1 and 2 h later. OTMs are the mean of 300 nuclei, and error bars represent the standard errors of the means from three independent experiments, $n = 3$. Sham/MF exposures were for 24 h, and the flux density was 100 $\mu T_{RMS}$.

the level of menadione-induced ROS, observed consistently in measurements performed 30 min to 1 h later. This suggests the existence of MF responses independent of altered *CRY* expression. It has been pointed out that studies reporting MF-induced changes in *CRY* expression cannot distinguish between cryptochromes as magnetic detectors *vs.* downstream components in magnetic signal transduction (Juutilainen et al. 2018).

The low-magnitude upregulation of oxidative stress-inducible genes observed immediately at the end of MF exposure might explain the small reduction in the level of menadione-induced ROS seen 30–60 min after MF exposure. The upregulated genes were found to be antioxidant genes, and the majority of them have been reported to be involved in mitigating the levels of induced ROS production (Yoshida et al. 2001; Cortes-Wanstreet et al. 2009; Mailloux and Harper 2011; Bertuchi et al. 2012; Hou et al. 2015; Park 2020).

In the present study, no statistically significant effect was observed in 29 out of 32 of ROS/$O_2^{\bullet-}$ measurements, while a small but consistent decrease was observed in menadione-induced ROS (statistically significant only for the 50 Hz MF exposure). These findings are in contrast to the majority of previous studies, including those from our group, that reported increased ROS/$O_2^{\bullet-}$ levels in cells exposed to ELF MFs (Luukkonen et al. 2014; Kesari et al. 2015, 2016; Höytö et al. 2017; also see comprehensive reviews of MF effects on oxidative stress parameters: Wang and Zhang 2017; Schuermann and Mevissen 2021). However, there are some reports of ROS/$O_2^{\bullet-}$ reduction in response to MF exposure (Zmyslony et al. 2004; Patruno et al. 2010; Song et al. 2018). The contrasting findings from different research groups may be related to differences in biological models, experimental protocols, MF exposure duration and intensity, and the time points at which ROS/$O_2^{\bullet-}$ levels were assayed. However, it is more difficult to explain the differences between the present study and our previous studies using similar (but not identical) experimental designs and biological models. The most influential difference between this and our earlier studies may be the different incubation times between MF exposure and measurement. Measurements of ROS and $O_2^{\bullet-}$ were performed in the present study 30–60 min after MF exposure, but generally after a 3-h incubation (with or without menadione) in our previous studies. In measurements done without the 3-h incubation, no MF effects on ROS, reduced glutathione, or lipid peroxidation were observed (Luukkonen et al. 2014). Other possible explanations may include different pre-experiment physiological conditions of the cells and minor differences in the geomagnetic field, which may be an essential variable in biological responses to ELF MFs (Naarala et al. 2017).

Differences in DNA damage-signaling gene expression were marginal and unpatterned, and the number of statistically significant differences between experimental conditions did not show marked deviations from the expected number of false-positive findings. This apparent lack of effects is consistent with the finding that no significant effects were found on DNA damage level or DNA damage repair rate. In a previous study, we found that 24-h exposure to 50 Hz, 200 μT MFs reduced the repair rate of DNA damage induced by

1-h treatment of murine FDC-P1 cells with 20 μ/ml bleomycin, leading to an elevated DNA damage level in the MF-exposed cells at the end of the time (2 h) allowed for repair (Mustafa et al. 2021). In an earlier study from our group, 24-h exposure of human SH-SY5Y to 50 Hz, 100 μT MFs increased the level of DNA damage immediately after a 3-h treatment with 20 μM menadione, but also increased repair rate during early (measured at 7.5 and 15 min) DNA repair, and impaired the fidelity of repair, as indicated by increased post-repair micronucleus level in the MF-exposed cells (Luukkonen et al. 2011). Although the present study did not confirm disruption of DNA repair, the findings of this and the earlier studies are not necessarily contradictory; the divergent results may be related to the different cell lines, different flux densities, and different chemical agents used by Mustafa et al. (2021) and the different experimental protocol used by Luukkonen et al. (2011).

One of the main purposes of the present work was to examine possible differences in cellular responses to 50 and 60 Hz MFs. Identifying frequency-specific effects would help to develop hypotheses of plausible mechanisms that could explain the purported effects of weak ELF MFs in the presence of the geomagnetic field (Juutilainen et al. 2018). In the present study, no firm evidence was found for distinct frequency-dependent effects. Although the decrease in menadione-induced ROS was statistically significant only in cells exposed to the 50 Hz MFs, a small but consistent difference in the same direction was also seen after exposure to 60 Hz MFs. The possible non-specific, low-magnitude increase in the expression of antioxidant genes immediately after exposure was associated with both 50 and 60 Hz exposures. The genes showing statistically significant changes were different for the 50 and 60 Hz experiments, but chance, as discussed above, is a plausible explanation for this observation. The only gene that showed a potential difference in responses to 50 and 60 Hz fields was *PRNP*, which showed a nearly 2-fold increase after the 50 Hz exposure but no change after the 60 Hz exposure.

The effect size of all statistically significant differences observed in this study was small. This is consistent with many previous studies that have reported effects on ROS-related endpoints and supports the hypothesis that MF exposure affects ROS signaling rather than causes oxidative stress (Juutilainen et al. 2018). Real-time monitoring of ROS levels would be valuable in future studies to increase understanding of what happens during MF exposure. It would also be worth looking at frequencies other than 50 and 60 Hz (but around them) to further evaluate possible frequency-dependent effects. Although no good candidates for magnetoresponsive genes were identified, it may be informative to carry out further testing of *HMOX1* as a possible MF-responsive gene and *PRNP* as a gene that might respond in a frequency-specific manner.

## Conclusion

Magnetic field exposure was found to cause at most small, if any, changes in gene expression. The tendency toward general low-magnitude upregulation of ROS-inducible genes immediately after MF exposure, however, may indicate adaptation to a small increase in ROS level. Such an upregulation would fit with the observed reduction of menadione-induced ROS assayed 30–60 min after MF exposure. MF exposures were not found to affect cytosolic $O_2^{\bullet-}$ levels, DNA damage or its repair rate, or gene expression related to DNA damage signaling. No firm evidence was found for differential effects from 50 and 60 Hz MFs, but further studies on frequency-dependence of ELF MF effects are definitely needed. Overall, the size of any MF effects was small, suggesting effects on ROS signaling rather than induction of oxidative stress.

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**Online Resources**

Supplementary Table 1. Human oxidative stress genes. PAHS-065YA RT$^2$ Profiler PCR Array (QIAGEN Sciences, Maryland, USA).

| Gene | Accession number | Description |
|---|---|---|
| *AKR1C2* | NM_001354 | Aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid-binding protein; 3-alpha hydroxysteroid dehydrogenase, type III) |
| *ALB* | NM_000477 | Albumin |
| *ALOX12* | NM_000697 | Arachidonate 12-lipoxygenase |
| *AOX1* | NM_001159 | Aldehyde oxidase 1 |
| *APOE* | NM_000041 | Apolipoprotein E |
| *ATOX1* | NM_004045 | ATX1 antioxidant protein 1 homolog (yeast) |
| *BAG2* | NM_004282 | BCL2-associated athanogene 2 |
| *BNIP3* | NM_004052 | BCL2/adenovirus E1B 19kDa interacting protein 3 |
| *CAT* | NM_001752 | Catalase |
| *CCL5* | NM_002985 | Chemokine (C-C motif) ligand 5 |
| *CCS* | NM_005125 | Copper chaperone for superoxide dismutase |
| *CYBB* | NM_000397 | Cytochrome b-245, beta polypeptide |
| *CYGB* | NM_134268 | Cytoglobin |
| *DHCR24* | NM_014762 | 24-dehydrocholesterol reductase |
| *DUOX1* | NM_175940 | Dual oxidase 1 |
| *DUOX2* | NM_014080 | Dual oxidase 2 |
| *DUSP1* | NM_004417 | Dual specificity phosphatase 1 |
| *EPHX2* | NM_001979 | Epoxide hydrolase 2, cytoplasmic |
| *EPX* | NM_000502 | Eosinophil peroxidase |
| *FHL2* | NM_001450 | Four and a half LIM domains 2 |
| *FOXM1* | NM_021953 | Forkhead box M1 |
| *FTH1* | NM_002032 | Ferritin, heavy polypeptide 1 |
| *GCLC* | NM_001498 | Glutamate-cysteine ligase, catalytic subunit |
| *GCLM* | NM_002061 | Glutamate-cysteine ligase, modifier subunit |
| *GLA* | NM_000169 | Galactosidase, alpha |
| *GPX1* | NM_000581 | Glutathione peroxidase 1 |
| *GPX2* | NM_002083 | Glutathione peroxidase 2 (gastrointestinal) |
| *GPX3* | NM_002084 | Glutathione peroxidase 3 (plasma) |
| *GPX4* | NM_002085 | Glutathione peroxidase 4 (phospholipid hydroperoxidase) |
| *GPX5* | NM_001509 | Glutathione peroxidase 5 (epididymal androgen-related protein) |

Supplementary Table 1. Continued

| Gene | Accession number | Description |
|---|---|---|
| *GSR* | NM_000637 | Glutathione reductase |
| *GSS* | NM_000178 | Glutathione synthetase |
| *GSTP1* | NM_000852 | Glutathione S-transferase pi 1 |
| *GSTZ1* | NM_001513 | Glutathione transferase zeta 1 |
| *HMOX1* | NM_002133 | Heme oxygenase (decycling) 1 |
| *HSP90AA1* | NM_001017963 | Heat shock protein 90kDa alpha (cytosolic), class A member 1 |
| *HSPA1A* | NM_005345 | Heat shock 70kDa protein 1A |
| *KRT1* | NM_006121 | Keratin 1 |
| *LHPP* | NM_022126 | Phospholysine phosphohistidine inorganic pyrophosphate phosphatase |
| *LPO* | NM_006151 | Lactoperoxidase |
| *MB* | NM_005368 | Myoglobin |
| *MBL2* | NM_000242 | Mannose-binding lectin (protein C) 2, soluble |
| *MPO* | NM_000250 | Myeloperoxidase |
| *MPV17* | NM_002437 | MpV17 mitochondrial inner membrane protein |
| *MSRA* | NM_012331 | Methionine sulfoxide reductase A |
| *MT3* | NM_005954 | Metallothionein 3 |
| *NCF1* | NM_000265 | Neutrophil cytosolic factor 1 |
| *NCF2* | NM_000433 | Neutrophil cytosolic factor 2 |
| *NCOA7* | NM_181782 | Nuclear receptor coactivator 7 |
| *NOS2* | NM_000625 | Nitric oxide synthase 2, inducible |
| *NOX4* | NM_016931 | NADPH oxidase 4 |
| *NOX5* | NM_024505 | NADPH oxidase, EF-hand calcium binding domain 5 |
| *NQO1* | NM_000903 | NAD(P)H dehydrogenase, quinone 1 |
| *NUDT1* | NM_002452 | Nudix (nucleoside diphosphate linked moiety X)-type motif 1 |
| *PDLIM1* | NM_020992 | PDZ and LIM domain 1 |
| *PRDX1* | NM_002574 | Peroxiredoxin 1 |
| *PRDX2* | NM_005809 | Peroxiredoxin 2 |
| *PRDX3* | NM_006793 | Peroxiredoxin 3 |
| *PRDX4* | NM_006406 | Peroxiredoxin 4 |
| *PRDX5* | NM_181652 | Peroxiredoxin 5 |
| *PRDX6* | NM_004905 | Peroxiredoxin 6 |

Supplementary Table 1. Continued

| Gene | Accession number | Description |
|---|---|---|
| *PRNP* | NM_183079 | Prion protein |
| *PTGR1* | NM_012212 | Prostaglandin reductase 1 |
| *PTGS1* | NM_000962 | Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) |
| *PTGS2* | NM_000963 | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) |
| *RNF7* | NM_014245 | Ring finger protein 7 |
| *SEPP1* | NM_005410 | Selenoprotein P, plasma, 1 |
| *SFTPD* | NM_003019 | Surfactant protein D |
| *SIRT2* | NM_012237 | Sirtuin 2 |
| *SLC7A11* | NM_014331 | Solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11 |
| *SOD1* | NM_000454 | Superoxide dismutase 1, soluble |
| *SOD2* | NM_000636 | Superoxide dismutase 2, mitochondrial |
| *SOD3* | NM_003102 | Superoxide dismutase 3, extracellular |
| *SPINK1* | NM_003122 | Serine peptidase inhibitor, Kazal type 1 |
| *SQSTM1* | NM_003900 | Sequestosome 1 |
| *SRXN1* | NM_080725 | Sulfiredoxin 1 |
| *TPO* | NM_000547 | Thyroid peroxidase |
| *TRAPPC6A* | NM_024108 | Trafficking protein particle complex 6A |
| *TTN* | NM_003319 | Titin |
| *TXN* | NM_003329 | Thioredoxin |
| *TXNRD1* | NM_003330 | Thioredoxin reductase 1 |
| *TXNRD2* | NM_006440 | Thioredoxin reductase 2 |
| *UCP2* | NM_003355 | Uncoupling protein 2 (mitochondrial, proton carrier) |
| *VIMP* | NM_203472 | Selenoprotein S |
| Reference genes | | |
| *ACTB* | NM_001101 | Actin, beta |
| *B2M* | NM_004048 | Beta-2-microglobulin |
| *GAPDH* | NM_002046 | Glyceraldehyde-3-phosphate dehydrogenase |
| *HPRT1* | NM_000194 | Hypoxanthine phosphoribosyltransferase 1 |
| *RPLP0* | NM_001002 | Ribosomal protein, large, P0 |

Supplementary Table 2. Human DNA damage signaling genes. PAHS-029ZA RT$^2$ Profiler PCR Array (QIAGEN Sciences, Maryland, USA).

| Gene | Accession number | Description |
|---|---|---|
| *ABL1* | NM_005157 | C-abl oncogene 1, non-receptor tyrosine kinase |
| *APEX1* | NM_080649 | APEX nuclease (multifunctional DNA repair enzyme) 1 |
| *ATM* | NM_000051 | Ataxia telangiectasia mutated |
| *ATR* | NM_001184 | Ataxia-telangiectasia and Rad3 related |
| *ATRIP* | NM_032166 | ATR interacting protein |
| *ATRX* | NM_000489 | Alpha thalassemia/mental retardation syndrome X-linked |
| *BARD1* | NM_000465 | BRCA1 associated RING domain 1 |
| *BAX* | NM_004324 | BCL2-associated X protein |
| *BBC3* | NM_014417 | BCL2 binding component 3 |
| *BLM* | NM_000057 | Bloom syndrome, RecQ helicase-like |
| *BRCA1* | NM_007294 | Breast cancer 1, early onset |
| *BRIP1* | NM_032043 | BRCA1 interacting protein C-terminal helicase 1 |
| *CDC25A* | NM_001789 | Cell division cycle 25 homolog A (S. pombe) |
| *CDC25C* | NM_001790 | Cell division cycle 25 homolog C (S. pombe) |
| *CDK7* | NM_001799 | Cyclin-dependent kinase 7 |
| *CDKN1A* | NM_000389 | Cyclin-dependent kinase inhibitor 1A (p21, Cip1) |
| *CHEK1* | NM_001274 | CHK1 checkpoint homolog (S. pombe) |
| *CHEK2* | NM_007194 | CHK2 checkpoint homolog (S. pombe) |
| *CIB1* | NM_006384 | Calcium and integrin binding 1 (calmyrin) |
| *CRY1* | NM_004075 | Cryptochrome 1 (photolyase-like) |
| *CSNK2A2* | NM_001896 | Casein kinase 2, alpha prime polypeptide |
| *DDB1* | NM_001923 | Damage-specific DNA binding protein 1, 127kDa |
| *DDB2* | NM_000107 | Damage-specific DNA binding protein 2, 48kDa |
| *DDIT3* | NM_004083 | DNA-damage-inducible transcript 3 |
| *ERCC1* | NM_001983 | Excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence) |
| *ERCC2* | NM_000400 | Excision repair cross-complementing rodent repair deficiency, complementation group 2 |
| *EXO1* | NM_130398 | Exonuclease 1 |
| *FANCA* | NM_000135 | Fanconi anemia, complementation group A |
| *FANCD2* | NM_033084 | Fanconi anemia, complementation group D2 |
| *FANCG* | NM_004629 | Fanconi anemia, complementation group G |
| *FEN1* | NM_004111 | Flap structure-specific endonuclease 1 |

Supplementary Table 2. Continued

| Gene | Accession number | Description |
|---|---|---|
| *GADD45A* | NM_001924 | Growth arrest and DNA-damage-inducible, alpha |
| *GADD45G* | NM_006705 | Growth arrest and DNA-damage-inducible, gamma |
| *H2AFX* | NM_002105 | H2A histone family, member X |
| *HUS1* | NM_004507 | HUS1 checkpoint homolog (S. pombe) |
| *LIG1* | NM_000234 | Ligase I, DNA, ATP-dependent |
| *MAPK12* | NM_002969 | Mitogen-activated protein kinase 12 |
| *MBD4* | NM_003925 | Methyl-CpG binding domain protein 4 |
| *MCPH1* | NM_024596 | Microcephalin 1 |
| *MDC1* | NM_014641 | Mediator of DNA-damage checkpoint 1 |
| *MLH1* | NM_000249 | MutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) |
| *MLH3* | NM_014381 | MutL homolog 3 (E. coli) |
| *MPG* | NM_002434 | N-methylpurine-DNA glycosylase |
| *MRE11A* | NM_005590 | MRE11 meiotic recombination 11 homolog A (S. cerevisiae) |
| *MSH2* | NM_000251 | MutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) |
| *MSH3* | NM_002439 | MutS homolog 3 (E. coli) |
| *NBN* | NM_002485 | Nibrin |
| *NTHL1* | NM_002528 | Nth endonuclease III-like 1 (E. coli) |
| *OGG1* | NM_002542 | 8-oxoguanine DNA glycosylase |
| *PARP1* | NM_001618 | Poly (ADP-ribose) polymerase 1 |
| *PCNA* | NM_182649 | Proliferating cell nuclear antigen |
| *PMS1* | NM_000534 | PMS1 postmeiotic segregation increased 1 (S. cerevisiae) |
| *PMS2* | NM_000535 | PMS2 postmeiotic segregation increased 2 (S. cerevisiae) |
| *PNKP* | NM_007254 | Polynucleotide kinase 3'-phosphatase |
| *PPM1D* | NM_003620 | Protein phosphatase, Mg2+/Mn2+ dependent, 1D |
| *PPP1R15A* | NM_014330 | Protein phosphatase 1, regulatory (inhibitor) subunit 15A |
| *PRKDC* | NM_006904 | Protein kinase, DNA-activated, catalytic polypeptide |
| *RAD1* | NM_002853 | RAD1 homolog (S. pombe) |
| *RAD17* | NM_002873 | RAD17 homolog (S. pombe) |
| *RAD18* | NM_020165 | RAD18 homolog (S. cerevisiae) |
| *RAD21* | NM_006265 | RAD21 homolog (S. pombe) |
| *RAD50* | NM_005732 | RAD50 homolog (S. cerevisiae) |

Supplementary Table 2. Continued

| Gene | Accession number | Description |
|---|---|---|
| *RAD51* | NM_002875 | RAD51 homolog (S. cerevisiae) |
| *RAD51B* | NM_133509 | RAD51 homolog B (S. cerevisiae) |
| *RAD9A* | NM_004584 | RAD9 homolog A (S. pombe) |
| *RBBP8* | NM_002894 | Retinoblastoma binding protein 8 |
| *REV1* | NM_016316 | REV1 homolog (S. cerevisiae) |
| *RNF168* | NM_152617 | Ring finger protein 168 |
| *RNF8* | NM_183078 | Ring finger protein 8 |
| *RPA1* | NM_002945 | Replication protein A1, 70kDa |
| *SIRT1* | NM_012238 | Sirtuin 1 |
| *SMC1A* | NM_006306 | Structural maintenance of chromosomes 1A |
| *SUMO1* | NM_003352 | SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae) |
| *TOPBP1* | NM_007027 | Topoisomerase (DNA) II binding protein 1 |
| *TP53* | NM_000546 | Tumor protein p53 |
| *TP53BP1* | NM_005657 | Tumor protein p53 binding protein 1 |
| *TP73* | NM_005427 | Tumor protein p73 |
| *UNG* | NM_003362 | Uracil-DNA glycosylase |
| *XPA* | NM_000380 | Xeroderma pigmentosum, complementation group A |
| *XPC* | NM_004628 | Xeroderma pigmentosum, complementation group C |
| *XRCC1* | NM_006297 | X-ray repair complementing defective repair in Chinese hamster cells 1 |
| *XRCC2* | NM_005431 | X-ray repair complementing defective repair in Chinese hamster cells 2 |
| *XRCC3* | NM_005432 | X-ray repair complementing defective repair in Chinese hamster cells 3 |
| *XRCC6* | NM_001469 | X-ray repair complementing defective repair in Chinese hamster cells 6 |
| Reference genes | | |
| *ACTB* | NM_001101 | Actin, beta |
| *B2M* | NM_004048 | Beta-2-microglobulin |
| *GAPDH* | NM_002046 | Glyceraldehyde-3-phosphate dehydrogenase |
| *HPRT1* | NM_000194 | Hypoxanthine phosphoribosyltransferase 1 |
| *RPLP0* | NM_001002 | Ribosomal protein, large, P0 |

Supplementary Table 3. Oxidative stress-related genes showing statistically significant changes in response to menadione. Human SH-SY5Y neuroblastoma cells were sham-exposed or exposed for 24 h to 100 $\mu T_{RMS}$ MFs at 50 Hz or 60 Hz. After exposure, cells were incubated in menadione-containing or menadione-free media for 1 h, then assayed for gene expression. Fold changes (menadione treatment/no menadione treatment ± standard error of the mean) are from three independent experiments, n = 3. The values between the parentheses are the p-values.

| Gene | Sham 50 Hz | MF 50 Hz | Sham 60 Hz | MF 60 Hz |
|---|---|---|---|---|
| *AKR1C2* | -- | 1.310 ± 0.055 (0.023) | -- | -- |
| *ALOX12* | 2.089 ± 0.163 (0.012) | -- | 3.977 ± 0.710 (0.020) | -- |
| *AOX1* | 0.766 ± 0.044 (0.041) | 0.710 ± 0.056 (0.045) | -- | 0.645 ± 0.020 (0.005) |
| *APOE* | -- | -- | 2.612 ± 0.388 (0.023) | -- |
| *ATOX1* | 1.055 ± 0.010 (0.031) | -- | -- | -- |
| *BNIP3* | 1.236 ± 0.035 (0.017) | -- | 1.288 ± 0.062 (0.034) | 1.301 ± 0.040 (0.014) |
| *CAT* | 1.243 ± 0.012 (0.002) | 1.179 ± 0.029 (0.021) | -- | -- |
| *CCS* | -- | -- | 1.549 ± 0.128 (0.038) | -- |
| *CYGB* | 1.208 ± 0.041 (0.030) | -- | -- | -- |
| *DHCR24* | 1.045 ± 0.009 (0.039) | -- | -- | -- |
| *DUOX2* | 1.500 ± 0.131 (0.047) | -- | -- | -- |
| *DUSP1* | 5.090 ± 0.092 (0.000) | 4.891 ± 0.057 (0.000) | 3.871 ± 0.354 (0.004) | 3.822 ± 0.422 (0.008) |
| *FHL2* | 0.285 ± 0.027 (0.006) | 0.258 ± 0.019 (0.003) | 0.254 ± 0.028 (0.006) | 0.200 ± 0.014 (0.002) |
| *FOXM1* | 0.855 ± 0.020 (0.021) | -- | -- | -- |
| *GCLC* | 1.033 ± 0.005 (0.018) | -- | -- | -- |
| *GPX3* | -- | -- | 1.346 ± 0.071 (0.032) | -- |
| *GSR* | -- | -- | -- | 0.845 ± 0.032 (0.046) |
| *GSTP1* | -- | -- | -- | 1.109 ± 0.019 (0.026) |
| *HMOX1* | 2.950 ± 0.275 (0.008) | -- | 3.606 ± 0.430 (0.010) | 3.849 ± 0.854 (0.039) |
| *HSP90AA1* | -- | 1.273 ± 0.037 (0.015) | -- | -- |
| *HSPA1A* | 8.868 ± 1.029 (0.003) | 8.452 ± 1.451 (0.008) | 8.283 ± 2.461 (0.018) | 6.877 ± 1.557 (0.013) |
| *NCOA7* | -- | 0.838 ± 0.032 (0.042) | -- | -- |
| *PRDX1* | 1.131 ± 0.018 (0.017) | 1.181 ± 0.020 (0.011) | 1.345 ± 0.086 (0.047) | -- |
| *PRDX5* | -- | 1.120 ± 0.011 (0.008) | -- | 1.159 ± 0.025 (0.020) |
| *RNF7* | 1.215 ± 0.010 (0.002) | -- | 1.213 ± 0.019 (0.006) | 1.233 ± 0.059 (0.049) |
| *SLC7A11* | 0.817 ± 0.016 (0.010) | -- | -- | -- |
| *SQSTM1* | 1.290 ± 0.006 (0.000) | 1.262 ± 0.053 (0.032) | 1.354 ± 0.054 (0.018) | 1.280 ± 0.068 (0.043) |
| *TPO* | -- | 0.801 ± 0.040 (0.043) | -- | -- |
| *TXNRD1* | 0.827 ± 0.030 (0.036) | -- | -- | -- |
| *VIMP* | 1.264 ± 0.063 (0.044) | 1.240 ± 0.021 (0.006) | -- | -- |

Supplementary Table 4. DNA damage signaling-related genes showing statistically significant changes in response to menadione. Human SH-SY5Y neuroblastoma cells were sham-exposed or exposed for 24 h to 100 $\mu T_{RMS}$ MFs at 50 Hz or 60 Hz. After exposure, cells were incubated in menadione-containing or menadione-free media for 1 h, then assayed for gene expression. Fold changes (menadione treatment/no menadione treatment ± standard error of the mean) are from three independent experiments, n = 3. The values between the parentheses are the p-values.

| Gene | Sham 50 Hz | MF 50 Hz | Sham 60 Hz | MF 60 Hz |
|---|---|---|---|---|
| *APEX1* | 1.07 ± 0.009 (0.015) | -- | -- | -- |
| *ATR* | 1.221 ± 0.021 (0.007) | -- | -- | -- |
| *ATRIP* | 1.308 ± 0.055 (0.024) | -- | 1.435 ± 0.08 (0.025) | 1.271± 0.044 (0.021) |
| *ATRX* | 0.779 ± 0.045 (0.046) | 0.741 ± 0.046 (0.042) | -- | -- |
| *BARD1* | 0.801 ± 0.034 (0.035) | -- | -- | -- |
| *BAX* | -- | 1.096 ± 0.016 (0.025) | -- | -- |
| *BBC3* | 1.745 ± 0.131 (0.019) | 1.758 ± 0.106 (0.012) | -- | -- |
| *BRCA1* | 0.850 ± 0.022 (0.024) | -- | -- | -- |
| *CDC25A* | 1.344 ± 0.044 (0.012) | -- | -- | -- |
| *CDC25C* | 1.370 ± 0.060 (0.018) | -- | 1.333 ± 0.076 (0.036) | -- |
| *CDK7* | 1.258 ± 0.058 (0.037) | 1.237 ± 0.034 (0.017) | -- | -- |
| *CHEK2* | 1.490 ± 0.036 (0.004) | 1.427 ± 0.081 (0.025) | 1.550 ± 0.141 (0.043) | -- |
| *CIB1* | -- | 1.183 ± 0.022 (0.012) | -- | -- |
| *CSNK2A2* | -- | -- | -- | 1.438 ± 0.120 (0.047) |
| *DDB1* | 0.880 ± 0.018 (0.023) | 0.816 ± 0.028 (0.027) | -- | -- |
| *DDB2* | -- | -- | -- | 1.205 ± 0.032 (0.020) |
| *DDIT3* | 4.696 ± 0.589 (0.007) | 4.863 ± 0.563 (0.006) | 4.311 ± 0.494 (0.006) | 5.936 ± 1.111 (0.014) |
| *ERCC2* | 1.065 ± 0.009 (0.019) | 1.114 ± 0.045 (0.044) | -- | -- |
| *EXO1* | 1.142 ± 0.025 (0.026) | 1.157 ± 0.039 (0.049) | -- | -- |
| *FANCA* | 1.350 ± 0.071 (0.010) | 1.355 ± 0.090 (0.044) | -- | -- |
| *FANCD2* | 0.936 ± 0.008 (0.012) | -- | -- | -- |
| *FEN1* | 0.921 ± 0.010 (0.016) | 0.832 ± 0.010 (0.001) | -- | 0.814 ± 0.028 (0.026) |
| *GADD45G* | 6.214 ± 1.257 (0.011) | 6.281 ± 1.309 (0.013) | 6.738 ± 1.358 (0.015) | 5.613 ± 0.934 (0.011) |
| *H2AFX* | 1.560 ± 0.113 (0.025) | 1.666 ± 0.120 (0.019) | 1.632 ± 0.078 (0.010) | 1.397 ± 0.103 (0.049) |
| *MAPK12* | 1.252 ± 0.052 (0.033) | -- | 1.358 ± 0.074 (0.032) | 1.506 ± 0.116 (0.037) |
| *MCPH1* | -- | -- | 1.133 ± 0.012 (0.007) | -- |
| *MDC1* | 0.858 ± 0.015 (0.012) | -- | -- | -- |
| *MSH2* | -- | 0.834 ± 0.034 (0.047) | 0.860 ± 0.029 (0.045) | -- |
| *NTHL1* | 1.126 ± 0.021 (0.023) | -- | -- | -- |
| *OGG1* | -- | -- | -- | 1.514 ± 0.074 (0.013) |
| *PARP1* | -- | 0.738 ± 0.003 (0.021) | -- | -- |
| *PMS2* | -- | -- | 1.230 ± 0.041 (0.026) | -- |
| *PNKP* | 1.270 ± 0.063 (0.042) | 1.282 ± 0.015 (0.002) | -- | -- |
| *PPM1D* | 1.524 ± 0.073 (0.013) | 1.487 ± 0.122 (0.043) | 1.360 ± 0.066 (0.025) | 1.381 ± 0.046 (0.010) |
| *PPP1R15A* | 3.840 ± 0.185 (0.001) | 3.514 ± 0.518 (0.014) | 3.429 ± 0.376 (0.008) | 2.894 ± 0.220 (0.005) |
| *PRKDC* | 0.721 ± 0.036 (0.024) | 0.685 ± 0.055 (0.042) | -- | -- |
| *RAD17* | 0.844 ± 0 (<0.001) | 0.763 ± 0.042 (0.039) | -- | -- |
| *RAD18* | 1.393 ± 0.004 (<0.001) | 1.419 ± 0.064 (0.016) | 1.462 ± 0.047 (0.007) | -- |
| *RAD21* | -- | -- | -- | 0.890 ± 0.013 (0.016) |
| *RAD51* | 1.140 ± 0.023 (0.023) | 1.183 ± 0.028 (0.019) | -- | -- |
| *RAD51B* | 1.723 ± 0.149 (0.024) | 1.971 ± 0.168 (0.015) | 1.925 ± 0.278 (0.043) | -- |
| *RAD9A* | 1.492 ± 0.063 (0.011) | -- | 1.749 ± 0.197 (0.045) | -- |
| *RBBP8* | 0.936 ± 0.010 (0.027) | -- | -- | -- |

Supplementary Table 4. Continued

| Gene | Sham 50 Hz | MF 50 Hz | Sham 60 Hz | MF 60 Hz |
|---|---|---|---|---|
| *REV1* | 1.206 ± 0.042 (0.034) | 1.184 ± 0.042 (0.043) | -- | -- |
| *RNF168* | 1.178 ± 0.005 (0.001) | -- | -- | -- |
| *RNF8* | 1.228 ± 0.003 (<0.001) | 1.253 ± 0.020 (0.005) | 1.217 ± 0.011 (0.002) | 1.368 ± 0.031 (0.005) |
| *RPA1* | -- | 0.868 ± 0.002 (0.030) | -- | -- |
| *SIRT1* | 1.158 ± 0.022 (0.017) | -- | -- | -- |
| *SMC1A* | 0.734 ± 0.033 (0.021) | 0.788 ± 0.048 (0.021) | 0.780 ± 0.019 (0.010) | 0.854 ± 0.022 (0.025) |
| *TP53* | -- | -- | -- | 0.863 ± 0.005 (0.002) |
| *TP53BP1* | 0.854 ± 0.015 (0.013) | 0.819 ± 0.038 (0.049) | -- | -- |
| *XPC* | -- | -- | 1.213 ± 0.064 (0.021) | -- |
| *XRCC2* | 1.533 ± 0.089 (0.017) | 1.766 ± 0.166 (0.025) | 1.686 ± 0.119 (0.017) | -- |
| *XRCC3* | 1.307 ± 0.036 (0.007) | 1.352 ± 0.067 (0.025) | -- | -- |

# Chapter 5.

# General discussion

## 1 METHODOLOGICAL CONSIDERATIONS

Although bioelectromagnetics research has produced a large number of studies, results are inconsistent and sometimes contradictory, and no coherent picture of EMF bioeffects has been drawn. A source for the inconsistency could be the variation in the used experimental conditions (Regel and Achermann, 2011; Wood et al., 2016). However, it has been proposed that much of the inconsistency could be explained by the quality of the studies. Thus, five quality criteria have been named for producing reliable data on EMF bioeffects (Mattsson and Simkó, 2014; Simkó et al., 2016; Vijayalaxmi and Prihoda, 2019; Vijayalaxmi and Foster, 2021). These quality criteria are 1) sham exposure is present, 2) sample analysis is performed blindly, 3) dosimetry is adequately described, 4) the temperature is controlled, 5) positive control for the assayed endpoints is present. All of these quality criteria have been successfully met in the present study.

The cell lines used in the present study are relevant to the types of cancers proposed to be associated with exposure to EMF. For example, in chapter 2, we used primary astrocytes. Astrocytes represent ~50% of the cells in the brain (Brandao et al., 2019), and therefore they are a suitable model for glioma, the type of brain tumor proposed to be associated with RF radiation. In addition, the fact that these cells are primary cells is advantageous; they represent the normal cell status *in vivo*. However, using primary astrocytes was challenging, mainly because these cells grew slowly. This slow-growing character was a significant disadvantage, especially when performing the IGI experiments where long incubation periods (36 days in our experiments) are needed to assay genomic abnormalities in the progeny of exposed cells several generations after exposure. In chapter 3, we used murine myeloid FDC-P1 cells. These cells are hematopoietic progenitor stem cells that differentiate into monocytes. Disturbed monocyte production is one of the most common signs of childhood leukemia (Creutzig et al., 1987). Therefore, these cells are relevant to studying possible carcinogenic effects of ELF MFs. However, the use of these cells came with the limitation that they are interleukin 3 (IL-3)-dependent; they cannot grow in the absence of this growth factor. Purified IL-3 is expensive, and we had to produce it ourselves by growing another cell line (WEHI-3 cells). The culture media of WEHI-3 cells containing IL-3 was then collected and used to culture FDC-P1 cells.

Thermal effects are a possible source of artifact in bioelectromagnetic research (Vijayalaxmi and Prihoda, 2009, 2019; Luukkonen, 2011; Herrala, 2018; Vijayalaxmi and Foster, 2021). Thermal effects might result from the increased temperature of the samples due to the applied field or heating of the exposure system coils. These effects could particularly be a potential confounder when investigating possible effects of RF radiation. Here, in our RF experiments, cell cultures were continuously kept in isothermal conditions ($37.0 \pm 0.3$ °C) as the exposure system was equipped with a water-circulation system that allowed for heat exchange and compensating for any absorbed energy from the applied field.

Unlike the exposure system we used in ELF MFs experiments, a drawback of our RF exposure system was the limited number of samples it could accommodate. Each of the exposure chambers allowed only two Petri dishes at a time; thus, it was not possible to have more samples to perform DNA damage repair study or test the effect of RF fields on more than one concentration of the co-exposure chemical.

# 2 GENOTOXICITY, OXIDATIVE STRESS, CIRCADIAN RHYTHM, AND EMFs

## 2.1 SUMMARY OF THE FINDINGS

The present study explored possible effects of RF radiation on genotoxicity and genomic instability. In addition, the present study examined whether ELF MFs could affect oxidative or DNA-damage responses (including gene transcription related to these phenomena) and whether these fields could influence the transcription of circadian rhythm-related genes. To assess possible weak effects, assays were performed in response to sole EMF exposure or a combination of EMFs and chemicals known to induce genotoxicity or oxidative stress. Experiments conducted in the present study and their results are summarized in Table 1.

Table 1. Summary of the findings of the present study

| Assay | EMF exposure | Co-exposure | Outcome | Chapter |
|---|---|---|---|---|
| *Genotoxicity and induced genomic instability* | | | | |
| DNA damage, MN, and induced genomic stability in rat primary astrocytes | 872 MHz (GSM or CW), SAR 0.6 or 6 W/kg for 24 h | Menadione or MMS (after RF exposure) | Sole exposure to RF radiation had no effect. No consistent evidence was found for co-genotoxic effects of RF radiation and menadione or MMS. RF exposure had no impact on menadione-induced genomic instability. | 2 |
| DNA damage, DNA damage repair rate, and the transcription of DNA damage signaling-related genes in mouse FDC-P1 hematopoietic cells and human SH-SY5Y neuroblastoma cells | 50 or 60 Hz, 0.1 or 0.2 mT for 15 min, 2 h, 12 h, or 24 h | Bleomycin or menadione (after ELF MFs exposure) | Sole exposure to MFs had no effects on DNA damage. However, 24-h exposure to 50 Hz MFs at 0.2 mT impaired bleomycin-induced DNA damage repair rate in FDCP-1 cells. No such effect was observed in SH-SY5Y cells. MF effects on DNA damage signaling-related genes were sporadic and generally small. | 3 and 4 |
| *Oxidative stress* | | | | |
| ROS and cytosolic $O_2^{\bullet-}$ level, and the transcription of oxidative-stress related genes in human SH-SY5Y neuroblastoma cells | 50 or 60 Hz, 0.2 mT for 24 h | Menadione (after ELF MFs exposure) | Sole exposure to MFs had no effect. However, MFs resulted in a slight increase in the transcription of ROS-inducible genes assayed immediately after exposure. In addition, MFs slightly reduced ROS levels induced by menadione when assayed 30 min – 1 h after the exposure. | 4 |
| *Circadian rhythm* | | | | |
| Transcription of circadian rhythm-related genes in mouse FDC-P1 hematopoietic cells | 50 Hz, 0.2 mT for 15 min, 2 h, 12 h, or 24 h | None | Systematic change in the transcription of circadian rhythm-related genes after 12 and 24 of exposure. However, none of the affected genes was a core circadian gene (including the Cry*1* and *Cry2*, the genes encoding for cryptochromes; the magnetosensitive components of the circadian system). | 3 |
| Transcription of *CRY1* in human SH-SY5Y neuroblastoma cells | 50 or 60 Hz, 0.1 mT for 24 h | Menadione | ELF MFs showed no evidence for an effect on *CRY1* transcription in the absence or the presence of menadione. | 4 |

Cryptochrome-1 gene (*CRY1*), Cryptochrome-2 gene (*CRY2*), Continuous-wave (CW), Deoxyribonucleic acid (DNA), Extremely-low frequency (ELF), Global System for Mobile Communications (GSM), Hertz (Hz), Kilogram (Kg), Magnetic fields (MFs), Megahertz (MHz), Methyl methanesulfonate (MMS), Micronuclei (MN), Minute (min), Radiofrequency (RF), Reactive oxygen species (ROS), Specific absorption rate (SAR), Superoxide ($O_2^{\bullet-}$), Watt (W).

## 2.2 GENOTOXICITY, INDUCED GENOMIC INSTABILITY, AND EMFs

In the present study, sole exposure to EMFs consistently showed no effects on any of the assayed genotoxic endpoints. Mere RF exposure did not affect DNA damage or micronucleus frequency. This lack of impact was independent of SAR level or signal modulation. Likewise, sole exposure to ELF MFs did not affect DNA damage or its repair rate after any of the applied exposure durations. These findings might not be surprising. The experiments of the present study were designed to meet all of the pre-defined quality criteria of non-ionizing radiation biology research. It was previously suggested that when all of these quality criteria are met, the probability of reporting increased genetic damage from sole EMF exposure decreases, if not vanishes (Vijayalaxmi and Prihoda, 2009, 2019; Vijayalaxmi and Foster, 2021).

The present study results showed no consistent evidence for RF co-genotoxic effects. As discussed in chapter 2, it seemed plausible to interpret the three statistically significant modifications of the menadione- and methyl methanesulfonate-induced genotoxicity as chance findings. These few positive findings were not consistently found to be occurring in a particular direction. GSM-modulated signal decreased the Comet assay-estimated menadione-induced DNA damage at 0.6 W/kg but increased at 6 W/kg, whereas continuous-wave signal increased the frequency of methyl methanesulfonate-induced micronuclei at 0.6 W/kg and had no effect at 6 W/kg. Also, it is highly unlikely that the GSM-modulated signal would act only in combination with menadione and the continuous-wave signal only in combination with methyl methanesulfonate. The present study also examined possible induction of genomic instability by RF radiation. RF radiation was not found to cause induced genomic instability alone or combined with menadione. Overall, the present study findings are in line with previous reviews suggesting little evidence for RF radiation genotoxicity (Vijayalaxmi and Prihoda, 2008, 2012, 2019; Luukkonen, 2011; and Herrala 2018).

In the present study, the levels of chemically-induced DNA damage assayed immediately after chemical treatment were consistently unaffected by ELF MF exposure. ELF MF effects on DNA damage repair rate were inconsistent. Differences in the experimental protocols could explain such inconsistency. In chapter 3, ELF MFs impaired the repair rate of bleomycin-induced DNA damage in murine FDC-P1 hematopoietic cells only after a 24-h exposure to 200 μT MFs, while shorter exposures for 15 min, 2 h, or 12 h had no effect. Whereas, in chapter 4, exposing human SH-SY5Y neuroblastoma cells to MF for 24 h at 100 μT did not affect the repair rate of menadione-induced DNA damage. These inconsistent results also reflected the contradictory findings of earlier studies on ELF MF effect on DNA damage repair rate, where the experimental conditions differ significantly (Robison et al., 2002; Luukkonen et al., 2011; Giorgi et al., 2014; Mizuno et al., 2014; Woodbine et al., 2015). ELF MF effects on DNA damage-signaling genes were largely inconsistent and generally of small magnitude. Further studies are needed to clarify the biological significance of these changes, if any. However, a major challenge is that the kinetics of DNA damage responses at the molecular level are fast. The changes in the expression of DNA damage-involved genes could last only from seconds to minutes (Kochan et al., 2017). The rapidly evolving technology of live-microscopy imaging would provide an excellent tool to track the changes in mRNA/protein levels in real-time (Aymoz et al., 2016; Wu et al., 2019; Braselmann e al., 2020; Baladi et al., 2021).

## 2.3 OXIDATIVE STRESS AND ELF MFs

The present study results suggested an increase in the transcription of ROS-inducible genes when assayed immediately after MF exposure; this increase was seen after both 50 Hz and 60 Hz exposures. At first glance, this finding seems to contradict the reduction of menadione-induced reactive species levels consistently observed 30 min – 1 h after MF exposure. However, the upregulated genes were antioxidative and previously reported to mitigate chemically-induced ROS levels. This highlights the importance of using

different assays at several chronological time points to avoid misinterpretation of the results and draw a bigger picture of the sequence of changes caused by MF exposure. Variation in the timing of endpoint measurements could be one potential reason for the inconsistent results among studies investigating possible EMF biological effects.

In the present study, the observed effects on oxidative stress-related endpoints were of small size. This is in line with many previous studies that assayed the same or similar endpoints (WHO, 2007; Luukkonen, 2011; Wang and Zhang, 2017; Juutilanen et al., 2018; Schuermann and Mevissen, 2021) and suggests that MF effects are on ROS signaling rather than inducing oxidative stress. In future studies, it would be useful to live-image ROS to understand better what could be happening during the exposure.

## 2.4 CIRCADIAN RHYTHM AND ELF MFs

In the present study, ELF MFs showed no evidence for an effect on the transcription of core circadian rhythm genes, including *CRY1* and *CRY2* (the genes encoding for cryptochromes; the magnetosensitive components of the circadian system). However, the systematic change in the transcription of circadian rhythm-related genes observed after 12 h and 24 h of MF exposure is worth noting. In addition, the present findings on ROS level and DNA damage repair rate were independent of change in cryptochrome-gene transcription. These suggest that change in cryptochrome-gene transcription is not a prerequisite for MFs to influence downstream genes or cellular functions. It is also worth noting that a recent study investigating magnetosensitivity in HeLa cells has shown that MF effects on radical pair reactions in flavins are not bound to cryptochromes (Ikeya and Woodward, 2021).

The circadian rhythm-related gene *SLC9A3* is a good candidate gene for further research. This gene was the most affected by MF exposure in the present study. It showed the biggest fold change (2.875), and its rhythmic transcription was systematically modified by extending the exposure duration. *SLC9A3* (also known as *NHE3*: sodium–hydrogen antiporter 3) is a member of a family of membrane transporters that play an essential role in maintaining pH and cellular homeostasis and were found to be involved in DNA damage responses (Zhao et al., 2007; Goldman et al., 2010; Aredia et al., 2016).

# 3 CONCLUSIONS

The present study results consistently showed that sole exposure to EMFs caused no genotoxic effects. Co-genotoxic effects of RF radiation were inconsistent. RF radiation did not induce or enhance genomic instability. ELF MF effects on the chemically-induced DNA damage repair rate differed with differences in exposure duration, magnetic flux density, co-exposure chemical, and the used cell line.

There was suggestive evidence that ELF MFs resulted in a small increase in the transcription of ROS-inducible (antioxidant) genes assayed immediately after exposure. This finding would fit with the slight decrease of ROS level seen at several time points after MF exposure. The effect on any oxidative stress-related endpoint was small, suggesting ELF MF effects are on ROS signaling rather than inducing oxidative stress.

The present study found no evidence that ELF MFs could affect the transcription of core circadian rhythm genes, including the cryptochrome-encoding genes *CRY1* and *CRY2*. However, the systematic change in the transcription of circadian rhythm-related genes (up- then downregulation in response to prolonging the exposure duration) is worth noting and warrants further research.

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# Appendices

## Appendix 1.

### Methodology of reviewing the literature for genotoxic effects of RF radiation and ELF MFs

The literature was reviewed on possible genotoxic effects of RF radiations and ELF MFs using the University of Eastern Finland search service: UEF Primo (https://primo.uef.fi). This search service provides access to the published studies indexed in several international databases, such as EMBASE, PubMed, Scopus, and Web of Science. The search was limited to peer-reviewed articles published in 2018 or later in English. The following search query was used:

| | | |
|---|---|---|
| | Any field | 'magnetic field*' OR 'electromagnetic field*' OR 'magnetic radiation' OR 'electromagnetic radiation*' OR 'magnetic irradiation*' OR 'electromagnetic irradiation*' OR 'nonionizing* radiation*' OR 'non-ionizing* radiation*' OR 'non ionizing* radiation*' |
| AND | Any field | 'extremely low frequency' OR 'extremely low-frequency' OR 'low frequency' OR 'low-frequency' OR 'power frequency' OR 'power-frequency' OR 'radiofrequency' OR 'microwave*' OR 'millimeter*' OR 'mobile phon*' OR 'cell phon*' OR 'telephon*' |
| AND | Any field | 'genotoxic*' OR 'DNA damage' OR 'DNA repair' OR 'micronucle*' OR 'chromosom*' OR 'mutation*' OR 'aneuploid*' OR 'sister chromatid*' |

The search query reported 2323 studies. These reported studies, in addition to the accessible genotoxicity studies reported on the EMF-Portal literature database (https://www.emf-portal.org), were considered for the review. The studies were firstly screened based on title and abstract, then the full-text of potentially relevant articles were comprehensively reviewed.

## Appendix 2.

### Methodology of reviewing the literature for ELF MF effects on gene transcription

The literature was reviewed on possible effects of ELF MFs on gene transcription related to DNA damage signaling, oxidative stress, and circadian rhythm using UEF Primo. The search was limited to peer-reviewed studies published in English. The following search query was used:

| | | |
|---|---|---|
| | Any field | 'magnetic field*' OR 'electromagnetic field*' OR 'magnetic radiation' OR 'electromagnetic radiation*' OR 'magnetic irradiation*' OR 'electromagnetic irradiation*' OR 'nonionizing* radiation*' OR 'non-ionizing* radiation*' OR 'non ionizing* radiation*' |
| AND | Any field | 'extremely low frequency' OR 'extremely low-frequency' OR 'low frequency' OR 'low-frequency' OR 'power frequency' OR 'power-frequency' |
| AND | Any field | 'oxidative stress' OR 'oxidative' OR 'oxidant' OR 'oxidation' OR 'antioxidant' OR 'antioxidative' OR 'peroxidation' OR 'peroxidative' OR 'DNA damage' OR 'DNA damage signal*' OR 'DNA repair' OR 'DNA damage repair' OR 'circadian rhythm*' OR 'circadian clock*' OR 'biological clock*' |
| AND | Any field | 'expression' OR 'transcription' OR 'messenger RNA' OR 'mRNA' OR 'complementary DNA' OR 'cDNA' |

The search query reported 2819 studies. These reported studies, in addition to the accessible gene expression studies reported on the EMF-Portal literature database, were considered for the review. At first, the studies were screened based on title and abstract. Then, the full-text of potentially relevant articles were comprehensively reviewed and carefully looked up for the genes related to DNA damage signaling, oxidative stress, and circadian rhythm listed in tables 1, 2, and 3, respectively. The genes lists were primarily made based on the lists of genes of QIAGEN© RT2 Profiler PCR Arrays for pathway-specific gene expression of DNA damage signaling, oxidative stress, and circadian rhythm (cat. 330231).

The primary purpose of the present literature review was to explore the effects of ELF MFs on the pathway-specific gene transcription related to the above-mentioned pathways rather than investigating the effects on the global gene transcription. Thus, the reported microarray/transcriptomic studies were extensively reviewed. Results from these studies were included in the present literature review if they were confirmed by a gene-specific assay method such as quantitative/semi-quantitative polymerase chain reaction, fluorescence *in situ* hybridization, or northern blot.

Table 1. DNA damage signaling-related genes.

| Gene name | Other common names | Description |
|---|---|---|
| *ABL1* | | C-abl oncogene 1, non-receptor tyrosine kinase |
| *APEX1* | | APEX nuclease 1 (multifunctional DNA repair enzyme) |
| *ATM* | | Ataxia-telangiectasia mutated |
| *ATR* | | Ataxia-telangiectasia and Rad3 related |
| *ATRIP* | | ATR interacting protein |
| *ATRX* | | Alpha thalassemia/mental retardation syndrome X-linked |
| *BARD1* | | BRCA1 associated RING domain 1 |
| *BAX* | | BCL2-associated X protein |
| *BBC3* | *PUMA* | BCL2 binding component 3 |
| *BCL2* | | BCL2 apoptosis regulator |
| *BLM* | | Bloom syndrome, RecQ helicase-like |
| *BRCA1* | | BRCA1 DNA repair associated breast cancer gene |
| *BRCA2* | | BRCA2 DNA repair associated breast cancer gene |
| *BRIP1* | | BRCA1 interacting protein C-terminal helicase 1 |
| *CDC20* | | Cell division cycle 20 |
| *CDC25A* | | Cell division cycle 25A |
| *CDC25B* | | Cell division cycle 25B |
| *CDC25C* | | Cell division cycle 25C |
| *CDK7* | | Cyclin-dependent kinase 7 |
| *CDKN1A* | *Cip1, P21, P21CIP1, WAF1* | Cyclin-dependent kinase inhibitor 1A |
| *CHEK1* | | Checkpoint kinase 1 |
| *CHEK2* | *RAD53* | Checkpoint kinase 2 |
| *CIB1* | | Calcium and integrin binding 1 |
| *CSNK2A2* | | Casein kinase 2, alpha prime polypeptide |
| *DCLRE1A* | | DNA cross-link repair 1A |
| *DDB1* | | Damage-specific DNA binding protein 1, 127kDa |
| *DDB2* | | Damage-specific DNA binding protein 2, 48kDa |
| *DDIT3* | *GADD153, CHOP* | DNA-damage-inducible transcript 3 |
| *DDR48* | | DNA damage-responsive protein 48 (S. cerevisiae) |

Table 1. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *ERCC1* | | ERCC excision repair 1, endonuclease non-catalytic subunit |
| *ERCC2* | *XPD* | ERCC excision repair 2, TFIIH core complex helicase subunit |
| *EXO1* | | Exonuclease 1 |
| *FANCA* | | Fanconi anemia, complementation group A |
| *FANCC* | | Fanconi anemia, complementation group C |
| *FANCD2* | | Fanconi anemia, complementation group D2 |
| *FANCG* | | Fanconi anemia, complementation group G |
| *FEN1* | | Flap structure-specific endonuclease 1 |
| *GADD45A* | *GADD45* | Growth arrest and DNA-damage-inducible, alpha |
| *GADD45G* | | Growth arrest and DNA-damage-inducible, gamma |
| *H2AFX* | | H2A histone family, member X |
| *HUS1* | | HUS1 checkpoint clamp component |
| *LIG1* | | DNA ligase 1 |
| *LIG4* | | DNA ligase 4 |
| *MAPK12* | *P38GAMMA* | Mitogen-activated protein kinase 12 |
| *MBD4* | | Methyl-CpG binding domain protein 4 |
| *MCPH1* | | Microcephalin 1 |
| *MDC1* | | Mediator of DNA-damage checkpoint 1 |
| *MGMT* | *AGT* | O-6-methylguanine-DNA methyltransferase |
| *MIF* | | Macrophage migration inhibitory factor |
| *MLH1* | | MutL homolog 1, colon cancer, nonpolyposis type 2 |
| *MLH3* | | MutL homolog 3 |
| *MPG* | | N-methylpurine-DNA glycosylase |
| *MRE11A* | | MRE11 homolog, double-strand break repair nuclease |
| *MSH2* | | MutS homolog 2, colon cancer, nonpolyposis type 1 |
| *MSH3* | | MutS homolog 3 |
| *NBN* | *NBS1* | Nibrin |
| *NTHL1* | | Nth like DNA glycosylase 1 |
| *OGG1* | | 8-oxoguanine DNA glycosylase |

Table 1. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *PARP1* | *ADPRT1* | Poly (ADP-ribose) polymerase 1 |
| *PARP2* | | Poly (ADP-ribose) polymerase family, member 2 |
| *PCNA* | | Proliferating cell nuclear antigen |
| *PMS1* | | PMS1 homolog 1, mismatch repair system component |
| *PMS2* | | PMS1 homolog 2, mismatch repair system component |
| *PNKP* | | Polynucleotide kinase 3'-phosphatase |
| *POLD3* | | DNA polymerase delta 3, accessory subunit |
| *POLE* | | DNA polymerase epsilon, catalytic subunit |
| *POLH* | | DNA polymerase eta |
| *POLI* | | DNA polymerase Iota |
| *PPM1D* | *Wip1* | Protein phosphatase, Mg2+/Mn2+ dependent, 1D |
| *PPP1R15A* | *GADD34* | Protein phosphatase 1, regulatory (inhibitor) subunit 15A |
| *PRKDC* | *DNA-PKcs* | Protein kinase, DNA-activated, catalytic polypeptide |
| *PTTG1* | | Pituitary tumor-transforming gene 1 |
| *RAD1* | | RAD1 checkpoint DNA exonuclease |
| *RAD17* | | RAD17 checkpoint clamp loader component |
| *RAD18* | | RAD18 E3 ubiquitin-protein ligase |
| *RAD21* | | RAD21 cohesin complex component |
| *RAD50* | | RAD50 double-strand break repair protein |
| *RAD51* | | RAD51 recombinase |
| *RAD51B* | | RAD51 paralog B |
| *RAD51C* | | RAD51 paralog C |
| *RAD52* | | RAD52 homolog, DNA repair protein |
| *RAD9A* | | RAD9 checkpoint clamp component A |
| *RBBP8* | | Retinoblastoma binding protein 8 |
| *REV1* | | REV1 DNA directed polymerase |
| *RNF168* | | Ring finger protein 168 |
| *RNF8* | | Ring finger protein 8 |
| *RPA1* | | Replication protein A1, 70kDa |

Table 1. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *RTT107* | | Regulator of Ty1 transposition protein 107, BRCA homolog (S. cerevisiae) |
| *SIRT1* | | Sirtuin 1 |
| *SMC1A* | | Structural maintenance of chromosomes 1A |
| *SMC3* | | Structural maintenance of chromosomes 3 |
| *SUMO1* | | Small ubiquitin like modifier 1 |
| *TERF1* | | Telomeric repeat binding factor 1 |
| *TOPBP1* | | Topoisomerase (DNA) II binding protein 1 |
| *TP53* | *P53*, *Trp53* (mouse) | Tumor protein p53 |
| *TP53BP1* | *Trp53bp1* (mouse) | Tumor protein p53 binding protein 1 |
| *TP53INPI* | | Tumor protein p53 - induced nuclear protein 1 |
| *TP73* | | Tumor protein p73 |
| *UNG* | | Uracil-DNA glycosylase |
| *WRN* | | WRN RecQ like helicase, Werner syndrome |
| *WRNIP1* | | Werner helicase interacting protein 1 |
| *XPA* | | Xeroderma pigmentosum, complementation group A |
| *XPC* | | Xeroderma pigmentosum, complementation group C |
| *XRCC1* | | X-ray repair cross-complementing 1 |
| *XRCC2* | | X-ray repair cross-complementing 2 |
| *XRCC3* | | X-ray repair cross-complementing 3 |
| *XRCC4* | | X-ray repair cross-complementing 4 |
| *XRCC5* | *Ku80* | X-ray repair cross-complementing 5 |
| *XRCC6* | *Ku70* | X-ray repair cross-complementing 6 |

Table 2. Oxidative stress-related genes.

| Gene name | Other common names | Description |
|---|---|---|
| *ADCK3* | | AarF domain containing kinase 3 |
| *AKR1C2* | | Aldo-Keto Reductase Family 1 Member C2 |
| *ALB* | | Albumin |
| *ALOX12* | | Arachidonate 12-lipoxygenase |
| *AOX1* | | Aldehyde oxidase 1 |
| *APOE* | | Apolipoprotein E |
| *ATF6* | | Activating transcription factor 6 |
| *ATOX1* | | Antioxidant 1 Copper Chaperone |
| *BAG1* | | BCL2-associated athanogene |
| *BAG2* | | BCL2-associated athanogene 2 |
| *BAG3* | | BCL2-associated athanogene 3 |
| *BAG4* | | BCL2-associated athanogene 4 |
| *BAG5* | | BCL2-associated athanogene 5 |
| *BNIP3* | | BCL2/adenovirus E1B 19kDa interacting protein 3 |
| *CAT* | | Catalase |
| *CCL5* | *RANTES* | Chemokine (C-C motif) ligand 5 |
| *CCS* | | Copper chaperone for superoxide dismutase |
| *CCT2* | | Chaperonin containing TCP1, subunit 2 (beta) |
| *CCT3* | | Chaperonin containing TCP1, subunit 3 (gamma) |
| *CCT4* | | Chaperonin containing TCP1, subunit 4 (delta) |
| *CCT5* | | Chaperonin containing TCP1, subunit 5 (epsilon) |
| *CCT6A* | | Chaperonin containing TCP1, subunit 6A (zeta 1) |
| *CCT6B* | | Chaperonin containing TCP1, subunit 6B (zeta 2) |
| *CCT7* | | Chaperonin containing TCP1, subunit 7 (eta) |
| *CLPB* | *HSP78* | Caseinolytic Mitochondrial Matrix Peptidase Chaperone Subunit B |
| *COQ8A* | | Coenzyme Q8A |
| *CRYAA* | | Crystallin, alpha A |
| *CRYAB* | | Crystallin, alpha B |
| *CYBB* | | Cytochrome B-245 beta chain |

Table 2. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *CYGB* | | Cytoglobin |
| *DHCR24* | | 24-dehydrocholesterol reductase |
| *DNAJA1* | | DnaJ (Hsp40) homolog, subfamily A, member 1 |
| *DNAJA2* | | DnaJ (Hsp40) homolog, subfamily A, member 2 |
| *DNAJA3* | | DnaJ (Hsp40) homolog, subfamily A, member 3 |
| *DNAJA4* | | DnaJ (Hsp40) homolog, subfamily A, member 4 |
| *DNAJB1* | *HSP40* | DnaJ (Hsp40) homolog, subfamily B, member 1 |
| *DNAJB11* | | DnaJ (Hsp40) homolog, subfamily B, member 11 |
| *DNAJB12* | | DnaJ (Hsp40) homolog, subfamily B, member 12 |
| *DNAJB13* | | DnaJ (Hsp40) homolog, subfamily B, member 13 |
| *DNAJB14* | | DnaJ (Hsp40) homolog, subfamily B, member 14 |
| *DNAJB2* | | DnaJ (Hsp40) homolog, subfamily B, member 2 |
| *DNAJB5* | | DnaJ (Hsp40) homolog, subfamily B, member 5 |
| *DNAJB6* | | DnaJ (Hsp40) homolog, subfamily B, member 6 |
| *DNAJB7* | | DnaJ (Hsp40) homolog, subfamily B, member 7 |
| *DNAJB8* | | DnaJ (Hsp40) homolog, subfamily B, member 8 |
| *DNAJB9* | | DnaJ (Hsp40) homolog, subfamily B, member 9 |
| *DNAJC1* | | DnaJ (Hsp40) homolog, subfamily C, member 1 |
| *DNAJC10* | | DnaJ (Hsp40) homolog, subfamily C, member 10 |
| *DNAJC11* | | DnaJ (Hsp40) homolog, subfamily C, member 11 |
| *DNAJC12* | | DnaJ (Hsp40) homolog, subfamily C, member 12 |
| *DNAJC13* | | DnaJ (Hsp40) homolog, subfamily C, member 13 |
| *DNAJC14* | | DnaJ (Hsp40) homolog, subfamily C, member 14 |
| *DNAJC15* | | DnaJ (Hsp40) homolog, subfamily C, member 15 |
| *DNAJC16* | | DnaJ (Hsp40) homolog, subfamily C, member 16 |
| *DNAJC17* | | DnaJ (Hsp40) homolog, subfamily C, member 17 |
| *DNAJC18* | | DnaJ (Hsp40) homolog, subfamily C, member 18 |
| *DNAJC21* | | DnaJ (Hsp40) homolog, subfamily C, member 21 |
| *DNAJC3* | | DnaJ (Hsp40) homolog, subfamily C, member 3 |

Table 2. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *DNAJC5* | | DnaJ (Hsp40) homolog, subfamily C, member 5 |
| *DNAJC5B* | | DnaJ (Hsp40) homolog, subfamily C, member 5 beta |
| *DNAJC5G* | | DnaJ (Hsp40) homolog, subfamily C, member 5 gamma |
| *DNAJC6* | | DnaJ (Hsp40) homolog, subfamily C, member 6 |
| *DNAJC7* | | DnaJ (Hsp40) homolog, subfamily C, member 7 |
| *DNAJC8* | | DnaJ (Hsp40) homolog, subfamily C, member 8 |
| *DNAJC9* | | DnaJ (Hsp40) homolog, subfamily C, member 9 |
| *DUOX1* | | Dual oxidase 1 |
| *DUOX2* | | Dual oxidase 2 |
| *DUSP1* | *PTPN16* | Dual specificity phosphatase 1 |
| *EPHX2* | | Epoxide hydrolase 2, cytoplasmic |
| *EPX* | | Eosinophil peroxidase |
| *FHL2* | | Four and a half LIM domains 2 |
| *FOXM1* | | Forkhead box M1 |
| *FTH1* | | Ferritin, heavy polypeptide 1 |
| *GCLC* | *GCS Heavy Chain* | Glutamate-cysteine ligase, catalytic subunit |
| *GCLM* | *GSC Light Chain* | Glutamate-cysteine ligase, modifier subunit |
| *GLA* | | Galactosidase, alpha |
| *GPX1* | | Glutathione peroxidase 1 |
| *GPX2* | | Glutathione peroxidase 2 (gastrointestinal) |
| *GPX3* | | Glutathione peroxidase 3 (plasma) |
| *GPX4* | | Glutathione peroxidase 4 (phospholipid hydroperoxidase) |
| *GPX5* | | Glutathione peroxidase 5 (epididymal androgen-related protein) |
| *GSR* | *GR* | Glutathione reductase |
| *GSS* | | Glutathione synthetase |
| *GST* | | Glutathione S-transferase |
| *GSTM2* | | Glutathione S-transferase mu 2 |
| *GSTM3* | | Glutathione S-transferase mu 3 |
| *GSTO1* | | Glutathione S-transferase omega 1 |

Table 2. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *GSTP1* | | Glutathione S-transferase pi 1 |
| *GSTT1* | | Glutathione S-Transferase theta 1 |
| *GSTZ1* | | Glutathione transferase zeta 1 |
| *HMOX1* | *HO-1* | Heme oxygenase (decycling) 1 |
| *HSF1* | *TCF5* | Heat shock transcription factor 1 |
| *HSF4* | | Heat shock transcription factor 4 |
| *HSP16* | | Heat shock protein 26kDa (*Caenorhabditis elegans*) |
| *HSP26* | | Heat shock protein 26kDa (*Drosophila melanogaster*) |
| *HSP104* | | Heat shock protein 104kDa (yeast) |
| *HSP70A* | | Heat shock 70 kDa protein A (651 aa) (algae) |
| *HSP70C* | | Heat shock 70 kDa protein B (bacteria) |
| *HSP90AA1* | *HSP90, HSP90α* | Heat shock protein 90kDa alpha (cytosolic), class A member 1 |
| *HSP90AB1* | *HSP90β* | Heat shock protein 90kDa alpha (cytosolic), class B member 1 |
| *HSP90B1* | | Heat shock protein 90kDa beta (Grp94), member 1 |
| *HSPA14* | | Heat shock 70kDa protein 14 |
| *HSPA1A* | *HSP70-1A, HSP72* | Heat shock 70kDa protein 1A |
| *HSPA1B* | | Heat shock 70kDa protein 1B |
| *HSPA1L* | | Heat shock 70kDa protein 1-like |
| *HSPA2* | | Heat shock 70kDa protein 2 |
| *HSPA4* | *HSP70* | Heat shock 70kDa protein 4 |
| *HSPA4L* | *OSP94* | Heat shock 70kDa protein 4-like |
| *HSPA5* | *GRP78* | Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) |
| *HSPA6* | *HSP70B'* | Heat shock 70kDa protein 6 |
| *HSPA7* | *HSP70B* | Heat shock protein family A (Hsp70) member 7 (Pseudogene) |
| *HSPA8* | | Heat shock 70kDa protein 8 |
| *HSPA9* | | Heat Shock Protein Family A (Hsp70) Member 9 |
| *HSPB1* | *HSP27* | Heat shock 27kDa protein 1 |
| *HSPB2* | | Heat shock 27kDa protein 2 |
| *HSPB3* | | Heat shock 27kDa protein 3 |

Table 2. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *HSPB6* | | Heat shock protein, alpha-crystallin-related, B6 |
| *HSPB7* | | Heat shock 27kDa protein family, member 7 (cardiovascular) |
| *HSPB8* | *HSP22* | Heat shock 22kDa protein 8 |
| *HSPD1* | *HSP60* | Heat shock 60kDa protein 1 (chaperonin) |
| *HSPE1* | | Heat shock 10kDa protein 1 (chaperonin 10) |
| *HSPH1* | *HSP105* | Heat shock 105kDa/110kDa protein 1 |
| *KRT1* | | Keratin 1 |
| *LHPP* | | Phospholysine Phosphohistidine Inorganic Pyrophosphate Phosphatase |
| *LPO* | | Lactoperoxidase |
| *MB* | | Myoglobin |
| *MBL2* | | Mannose-binding lectin (protein C) 2, soluble |
| *MGST1* | | Microsomal glutathione S-transferase 1 |
| *MGST3* | | Microsomal glutathione S-transferase 3 |
| *MPO* | | Myeloperoxidase |
| *MPV17* | | MpV17 mitochondrial inner membrane protein |
| *MSRA* | | Methionine sulfoxide reductase A |
| *MT3* | | Metallothionein 3 |
| *NCF1* | | Neutrophil cytosolic factor 1 |
| *NCF2* | | Neutrophil cytosolic factor 2 |
| *NCOA7* | | Nuclear receptor coactivator 7 |
| *NFE2L2* | *NRF2* | Nuclear factor, erythroid 2 Like 2 |
| *NOS2* | *iNOS* | Nitric oxide synthase 2, inducible |
| *NOX4* | | NADPH oxidase 4 |
| *NOX5* | | NADPH oxidase, EF-hand calcium-binding domain 5 |
| *NQO1* | | NAD(P)H dehydrogenase, quinone 1 |
| *NQO2* | | NAD(P)H dehydrogenase, quinone 2 |
| *NUDT1* | | Nudix (nucleoside diphosphate linked moiety X)-type motif 1 |
| *PDLIM1* | | PDZ and LIM domain 1 |
| *PFDN1* | | Prefoldin subunit 1 |

Table 2. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *PFDN2* | | Prefoldin subunit 2 |
| *PRDX1* | | Peroxiredoxin 1 |
| *PRDX2* | | Peroxiredoxin 2 |
| *PRDX3* | | Peroxiredoxin 3 |
| *PRDX4* | | Peroxiredoxin 4 |
| *PRDX5* | | Peroxiredoxin 5 |
| *PRDX6* | *AOP2* | Peroxiredoxin 6 |
| *PRNP* | | Prion protein |
| *PTGR1* | | Prostaglandin reductase 1 |
| *PTGS1* | *COX1* | Prostaglandin-endoperoxide synthase 1 |
| *PTGS2* | *COX2* | Prostaglandin-endoperoxide synthase 2 |
| *RNF7* | | Ring finger protein 7 |
| *SELENOP* | *SEPP1* | Selenoprotein P, plasma, 1 |
| *SELENOS* | *VIMP* | Selenoprotein S |
| *SERPINH1* | *HSP47* | Serpin family H member 1 |
| *SFTPD* | | Surfactant protein D |
| *SIL1* | | SIL1 Nucleotide Exchange Factor |
| *SIRT2* | | Sirtuin 2 |
| *SLC7A11* | | Solute carrier family 7 member 11 |
| *SOD1* | | Superoxide dismutase 1, soluble |
| *SOD2* | | Superoxide dismutase 2, mitochondrial |
| *SOD3* | | Superoxide dismutase 3, extracellular |
| *SPINK1* | | Serine peptidase inhibitor, Kazal type 1 |
| *SQSTM1* | | Sequestosome 1 |
| *SRXN1* | | Sulfiredoxin 1 |
| *TCP1* | | T-complex 1 |
| *TOR1A* | | Torsin family 1, member A (torsin A) |
| *TPO* | | Thyroid peroxidase |
| *TRAP1* | *HSP75* | TNF receptor associated protein 1 |

Table 2. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *TRAPPC6A* | | Trafficking protein particle complex 6A |
| *TTN* | | Titin |
| *TXN* | | Thioredoxin |
| *TXNRD1* | | Thioredoxin reductase 1 |
| *TXNRD2* | | Thioredoxin reductase 2 |
| *UCP2* | | Uncoupling protein 2 (mitochondrial, proton carrier) |

Table 3. Circadian rhythm-related genes.

| Gene name | Other common names | Description |
|---|---|---|
| *AANAT* | | Aralkylamine N-acetyltransferase |
| *ALAS1* | | Aminolevulinate, delta-, synthase 1 |
| *ARNTL* | *BMAL1* | Aryl hydrocarbon receptor nuclear translocator-like |
| *ARNTL2* | | Aryl hydrocarbon receptor nuclear translocator-like 2 |
| *ATOH7* | | Atonal BHLH transcription factor 7 |
| *BHLHE40* | *DEC1* | Basic helix-loop-helix family, member e40 |
| *BHLHE41* | | Basic helix-loop-helix family, member e41 |
| *CAMK2A* | | Calcium/calmodulin-dependent protein kinase II alpha |
| *CAMK2B* | | Calcium/calmodulin-dependent protein kinase II beta |
| *CAMK2D* | | Calcium/calmodulin-dependent protein kinase II delta |
| *CAMK2G* | | Calcium/calmodulin-dependent protein kinase II gamma |
| *CARTPT* | | CART prepropeptide |
| *CCRN4L* | | CCR4 carbon catabolite repression 4-like (S. cerevisiae) |
| *CHRNB2* | | Cholinergic receptor nicotinic beta 2 subunit |
| *CLOCK* | | Clock circadian regulator |
| *CREB1* | | CAMP responsive element binding protein 1 |
| *CREB3* | | CAMP responsive element binding protein 3 |
| *CRX* | | Cone-rod homeobox |
| *CRY1* | | Cryptochrome circadian regulator 1 |
| *CRY2* | | Cryptochrome circadian regulator 2 |
| *CSNK1A1* | | Casein kinase 1, alpha 1 |
| *CSNK1D* | | Casein kinase 1, delta |
| *CSNK1E* | | Casein kinase 1, epsilon |
| *CSNK2A1* | | Casein kinase 2, alpha 1 polypeptide |
| *CSNK2A2* | | Casein kinase 2, alpha prime polypeptide |
| *DBP* | | D site of albumin promoter (albumin D-box) binding protein |
| *EGR1* | | Early growth response 1 |
| *EGR3* | | Early growth response 3 |
| *EPO* | | Erythropoietin |

Table 3. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *ESRRA* | | Estrogen related receptor alpha |
| *FBXL21* | | F-box and leucine-rich repeat protein 21 (gene/pseudogene) |
| *FBXL3* | | F-box and leucine-rich repeat protein 3 |
| *HEBP1* | | Heme binding protein 1 |
| *HLF* | | Hepatic leukemia factor |
| *HTR7* | | 5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled) |
| *IRF1* | | Interferon regulatory factor 1 |
| *KCNMA1* | | Potassium large conductance calcium-activated channel, subfamily M, alpha member 1 |
| *MAPK1* | | Mitogen-activated protein kinase 1 |
| *MAPK3* | | Mitogen-activated protein kinase 3 |
| *MAPK14* | *P38* | Mitogen-activated protein kinase 14 |
| *MAT2A* | | Methionine adenosyltransferase II, alpha |
| *MTNR1A* | | Melatonin receptor 1A |
| *MTNR1B* | | Melatonin receptor 1B |
| *MYOD1* | | Myogenic differentiation 1 |
| *NCOA3* | *AIB1* | Nuclear receptor coactivator 3 |
| *NFIL3* | | Nuclear factor, interleukin 3 regulated |
| *NKX2-5* | | NK2 homeobox 5 |
| *NMS* | | Neuromedin S |
| *NOCT* | | Nocturnin |
| *NPAS2* | | Neuronal PAS domain protein 2 |
| *NR1D1* | *REV-ERB* | Nuclear receptor subfamily 1, group D, member 1 |
| *NR1D2* | | Nuclear receptor subfamily 1, group D, member 2 |
| *NR2F6* | | Nuclear receptor subfamily 2, group F, member 6 |
| *OPN3* | | Opsin 3 |
| *OPN4* | | Opsin 4 |
| *PAX4* | | Paired box 4 |
| *PER1* | | Period circadian regulator 1 |
| *PER2* | | Period circadian regulator 2 |

Table 3. Continued

| Gene name | Other common names | Description |
|---|---|---|
| *PER3* | | Period circadian regulator 3 |
| *POU2F1* | | POU class 2 homeobox 1 |
| *PPARA* | | Peroxisome proliferator-activated receptor alpha |
| *PPARGC1A* | *PGC-1-Alpha* | Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha |
| *PRF1* | | Perforin 1 (pore-forming protein) |
| *PRKACA* | | Protein kinase CAMP-activated catalytic subunit alpha |
| *PRKACB* | | Protein kinase, cAMP-dependent, catalytic, beta |
| *PRKACG* | | Protein kinase, cAMP-dependent, catalytic, gamma |
| *PRKAR1A* | | Protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue-specific extinguisher 1) |
| *PRKAR1B* | | Protein kinase, cAMP-dependent, regulatory, type I, beta |
| *PRKAR2A* | | Protein kinase, cAMP-dependent, regulatory, type II, alpha |
| *PRKAR2B* | | Protein kinase, cAMP-dependent, regulatory, type II, beta |
| *PRKCA* | | Protein kinase C, alpha |
| *PRKCB* | | Protein kinase C, beta |
| *PROKR2* | | Prokineticin receptor 2 |
| *PTGDS* | | Prostaglandin D2 synthase 21kDa (brain) |
| *RORA* | | RAR-related orphan receptor A |
| *RORB* | | RAR-related orphan receptor B |
| *RORC* | | RAR-related orphan receptor C |
| *SLC9A3* | *NHE3* | Solute carrier family 9 (sodium/hydrogen exchanger), member 3 |
| *SMAD4* | *MADH4* | SMAD family member 4 |
| *SP1* | | Sp1 transcription factor |
| *SREBF1* | | Sterol regulatory element-binding transcription factor 1 |
| *STAT5A* | | Signal transducer and activator of transcription 5A |
| *TEF* | | Thyrotrophic embryonic factor |
| *TFAP2A* | | Transcription factor AP-2, alpha |
| *TGFB1* | | Transforming growth factor, beta 1 |
| *TIMELESS* | | Timeless circadian regulator |
| *WEE1* | | WEE1 G2 checkpoint Kinase |

# EHAB MUSTAFA

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Exposures to radiofrequency (RF) radiation and extremely low-frequency (ELF) magnetic fields have been suggested as possibly carcinogenic to humans. However, no plausible biophysical mechanisms have been established to explain the possible association between cancer and these exposures. At an *in vitro* level, the present doctoral dissertation examined possible effects of RF radiation and ELF magnetic fields on specific cancer-related phenomena: genotoxicity, genomic instability, oxidative stress, and circadian rhythm.

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