



**ANNA KOZIOROWSKA<sup>1</sup>, KATARZYNA STATS<sup>2</sup>,  
MARIA ROMEROWICZ-MISIELAK<sup>3</sup>**

## **The influence of environmental factors on metabolic activity of cancer cells**

<sup>1</sup> Doktor inżynier, Uniwersytet Rzeszowski, Wydział Matematyczno-Przyrodniczy, Katedra Inżynierii Komputerowej, Polska

<sup>2</sup> Inżynier, studentka, Uniwersytet Rzeszowski, Instytut Biotechnologii Stosowanej i Nauk Podstawowych, Katedra Fizjologii i Rozrodu Zwierząt, Polska

<sup>3</sup> Doktor, Uniwersytet Rzeszowski, Instytut Biotechnologii Stosowanej i Nauk Podstawowych, Katedra Fizjologii i Rozrodu Zwierząt, Polska

### **Abstract**

The paper presents the results of viability of breast cancer cells under the influence of electromagnetic field. MCF-7 cell line was subjected to an electromagnetic field with a frequency of 5 Hz, 60 Hz and 120 Hz and an MTT assay was performed immediately after the influence of the field and after 24 hours. No statistical difference was demonstrated in cell viability immediately after exposure to EMF, and there are demonstrated differences in the case of field frequencies of 5 Hz and 120 Hz within 24 hours after exposure.

**Key words:** cells viability, Electromagnetic field.

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### **Introduction**

Electromagnetic field (EMF) accompanies people in all areas of life. It is one of environmental factor which influence our everyday life. It is the space in which electric and magnetic energy permeate each other. These interrelationships are described by Maxwell's equations.

The electric field is directed perpendicular to the Earth. The value of the electric field strength depends on the weather conditions. During the good weather it is approx. 130 V/m, during dense fog to 2 kV/m, and during a storm it can be up to 20 kV/m. The magnetic field does not depend on atmospheric phenomena and its intensity reaches 40 A/m. [Siemiński 1994; Kudowski et al. 1997].

Sun and Earth produce the natural electromagnetic field and artificial fields are produced by a growing number of everyday devices. The sources of natural magnetic field are:

1. Geomagnetic field of the Earth (currently the magnetic south pole is located approx.  $7.3^\circ$  from the north pole of the Earth and the magnetic south pole of approx.  $27^\circ$  from the north pole of the Earth).

2. Atmospheric Phenomena (eg. Electrical discharges).
3. The fields coming from space (the most powerful source of the magnetic field is Jupiter and the solar radiation).
4. Ocean and sea tides.

The most widespread artificial sources of EMF are transmission lines and all household appliances supplied from industrial network. These devices are the source of the fields of extremely low frequency range of 50–60 Hz. Among the household appliances are also those which emit fields of higher frequencies, such as microwave ovens or mobile phones often worn in close proximity to the human body. These devices operate at high frequencies above 300 MHz.

Both – the electric the magnetic fields and are used in medicine. There are applied field of the entire frequency spectrum – 0–300 Hz. They are used in the diagnosis (eg. Magnetic resonance imaging), physiotherapy (terapuls, diathermy, treatment of fixed and modulated magnetic field) or interventional medicine (eg. Electrosurgical units).

EMF affecting the environment and human as any physical factor influence on living matter. The impact depends on the method of the field generating and from the time of exposure and frequency range. The electromagnetic field shows broad spectrum of action on living tissues. The mechanisms of these interactions are studied for many years, but are not fully understood.

Depending on the frequency, activity extends from stimulation of excitable tissues such as nerves, muscles and heart [Polk 1995; Palti,1966] by the stimulation of bone growth and accelerate of fracture healing [Besset, 1985] to use it for the thermal ablation of tumors using electromagnetic waves of a radio frequency. The electric field of an intermediate-frequency (> 10 kHz to MHz) was often considered as not exerting a biological effect [Elson 1995], and hence, medical application, a few non-thermal cell effects which has been observed [Zimmerman et al. 1981; Holzapfel et al. 1982; Pawlowski et al. 1993].

Until 1970 it was thought that exposure to electromagnetic fields is completely safe to people and does not lead to the formation of any damage or disrupt the functioning of tissues. Just in 1979, control clinical studies demonstrated the relationship between the place of residence in the vicinity of power networks with the number of cases of cancer [Wertheimer, Lepper 1979]. Since then, subsequent studies confirmed the relationship between raised risk of developing certain types of cancer, especially in children, and the constant staying in the vicinity power networks [London et al. 1991; Savitz et al. 1988; Feychting, Ahlbom 1993; Tomenius 1985].

One of the cases of examined cancer was breast cancer. The risk of breast cancer is significantly higher in the industrial urban areas, such as northern Europe and North America than in less developed areas, such as Africa and Asia [Stevens 1987; Stevens, Davis 1996]. In 1978, Cohen and his colleagues sug-

gested that the reduction of production of melatonin by the pineal gland can raise estrogen levels in the circulation, stimulate the proliferation of breast tissue and can lead to breast cancer [Cohen et al. 1978]. Cohen and colleagues hypothesized that the environmental light can be a factor which may lead to lower production of melatonin.

The last decade has brought a number of *in vitro* and *in vivo* studies, that have documented the antitumor effect of an alternating electric field [Kirson et al. 2007; Zimmerman et al. 2012], including the intermediate-frequency low-intensity (100–300 kHz) alternating electric field and the magnetic field amplitude-modulated slightly lower frequencies (0.1 Hz to 114 kHz) [Barbault et al. 2009].

Zimmermann et al. [2013] showed that the antitumor effect was achieved in a specific (for the type of tumor), frequency modulation, and demonstrated inhibition of proliferation and disruption of the mitotic spindle when exposed to an alternating electric field [Kirson et al. 2007; Zimmerman et al. 2012]. Moreover, the bridging important aspects of apoptosis [Fang et al. 1998; Silva et al. 1996] with an extremely low frequency (ELF) pulsed-gradient magnetic field, Zhang [Zhang et al. 2002] shows that can not only induce, but also can block the development of neovascularization required for the nutrition (blood supply to the tumor).

The hypothesis of a compound of the electromagnetic field with breast cancer is based on experimental evidence, that the light and electromagnetic fields of extremely low frequency affects the production of melatonin by the pineal gland, thus affecting the mammalian carcinogenesis in laboratory studies [Stevens 1987; Stevens, Davis 1996; Preston-Martin 1996]. Biological plausibility of the relationship between EMF and breast cancer is associated (conjugated) with unexplained high rate of breast cancer cases in some industrialized urban areas, suggesting that further investigations are warranted.

## **Materials and Methods**

### **Cell culture**

The research model used in this experiment was stabilized, derived from a human breast cancer cell line MCF-7. The cells were cultured in DMEM medium supplemented with 10% fetal calf serum (FBS). The culture was carried out at 37°C under 95% humidity and 5% CO<sub>2</sub> concentration in the air. All experiments were performed between 3 and 7 passage.

### **Exposure of MCF-7 cells to electromagnetic fields**

The dynamic magnetic field generator (Magneris, Astar) was used in this study. The distributions of EMF was determined by Astar using: magnetic field meter GM04 (Hirst Magnetic Instruments, UK), Hall effect sensor type A1321

(Allegro Microsystems), TDS1002B oscilloscope (Tektronix), BM515X digital multimeter (BRYMEN). Apparatus can generate low-frequency electromagnetic field in the range from 2 to 120 Hz with sinusoidal, triangular and rectangular shape. Magnetic field distribution inside the two-part flat applicator gives the opportunity to carry out tests for different values of magnetic induction (in the study from 2 to 6 mT). The shape of the magnetic field was directly dependent on the shape of the current passing through the solenoid (in the study sinusoidal shape, frequency 5 Hz, 60 Hz and 120 Hz).

Experiments were carried out on 96 well culture plates. 24 hours before the experiment, the MCF-7 cells were seeded on a plate at a density of 1000 cells/well. The cells were exposed to EMF by 2 hours. There were taken cells untreated by EMF as a control.

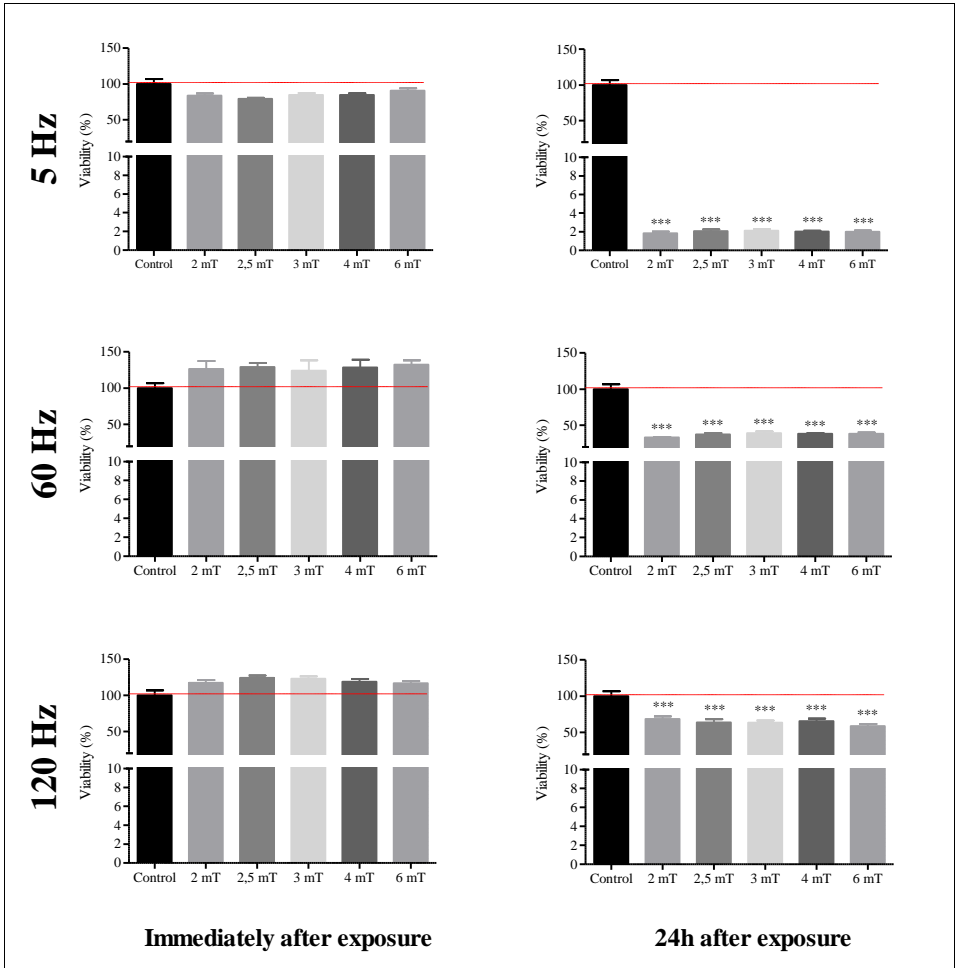
#### **Estimation of cell viability – MTT assay**

Metabolic activity was determined by quantitative colorimetric MTT assay (Sigma-Aldrich). The principle of the test is the reduction of yellow dissolved 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide to formazan blue, due to the action of succinate dehydrogenase. It is an enzyme functioning only in cells with intact mitochondrial oxidative activity, and therefore the MTT assay is used for the analysis of live cells [Dzitko et al. 2010].

The cells viability was estimated immediately after the two-hour exposure to an electromagnetic field after 24 hours. There was added a solution of 50µl of MTT reagent at concentration of 5 mg/ml to each well (Sigma-Aldrich; Niemcy). The cultures were incubated for 4 hours at 37°C, 95% humidity and at concentration of 5% CO<sub>2</sub> in the air. After incubation, the precipitated crystals of formazan were dissolved. The liquid from the crystal was removed by pipetting, and then 100 µl of solvent formazan (4 mM HCl, 0.1% Nanodet P-40 in isopropanol) was added to each well and gently stirred for 15 minutes to dissolve the crystals. The absorbance of the solution was measured spectrophotometrically at 492 nm using a Victor plate reader (Perkin Elmer). The results are shown as the percentage of viable cells in relation to the control.

## **Results**

Immediately after completion of the exposure to electromagnetic fields with frequency of 5 Hz, 60 Hz and 120 Hz there is not shown significant changes in MCF-7 cell viability. 24 hours after exposure to an electromagnetic field with a frequency of 5 Hz and at a magnetic induction of 2 to 6 mT, cells viability significantly decreased ( $P \leq 0.001$ ) in relation to the control. After 24 hours from the completion of the exposure to an electromagnetic field with a frequency of 120 Hz, there was a significant ( $P \leq 0.001$ ) increase in MCF-7 cell viability compared to the control. The results of studies are shown at Fig. 1.



**Fig. 1. The viability of MCF-7 breast cancer cells under the influence of electromagnetic field with frequency of 5 Hz, 60 Hz and 120 Hz immediately after exposure on EMF and 24 hours after exposure. Data are shown as the mean  $\pm$  SEM, \*\*\* $P \leq 0.001$**

## Discussion

In recent years greatly increased interest in the potential use of the electromagnetic field in anticancer therapy [Barbault et al. 2009; Blackman 2012; Cameron et al. 2005; Elson 2009; Zimmerman et al. 2012]. Numerous scientific reports concerning the particular sensitivity of tumor cells to electromagnetic fields of extremely low frequency contributed to this [Zimmerman et al. 2012, Crocetti et al. 2011; Ruiz-Gómez, Martinez-Morillo 2005; Yamaguchi et al. 2006]. At the same time exposure to ELF-EMF was found to be neutral or even beneficial to normal cells [Elson 2009; Repacholi, Greenebaum 1999]. The aim

of this study was to evaluate the effect of electromagnetic fields of extremely low frequency on the viability of MCF-7 breast cancer cells. The analysis was carried out *in vitro*. After 24-hour of incubation, cells were exposed to an electromagnetic field with a frequency of 5 Hz, 60 Hz and 120 Hz and a magnetic induction of 2–6 mT. Cells viability was estimated immediately after completion of exposure to EMF and after 24 hours.

There is demonstrated the significant effect of the electromagnetic field in the studied range of frequencies on the viability of MCF-7 cells. After 24 hours of incubation after the end of 2-hour exposure to EMF, breast cancer cells viability, measured by the MTT assay, was significantly lower in relation to the control (Fig. 1). The effectiveness of electromagnetic fields was dependent on their frequencies, there were no significant differences related to the value of a magnetic induction (2–6 mT). The highest decrease in viability of MCF-7 cells were observed for cell exposure to EMF with frequency of 5 Hz. The similar test results obtained Crocetti et al. [2013], showing the sensitivity of MCF-7 breast cancer cells, measured by the rate of proliferation to an electromagnetic field in the parameters of EMF and exposure time dependent manner. At the same time they did not observe the effect of the cytotoxic effects of EMF on the healthy breast cells (MCF-10). In the scientific papers there are number of studies proving the tumor cell proliferation inhibitory action of the electromagnetic field with the frequency range from 20 Hz to 100 Hz [Buckner et al. 2015; Zhang et al. 2012; Yan et al. 2010; Crocetti et al. 2013]. Studies on the biological effects of electromagnetic fields action relate mainly to its frequency of 50 Hz and 60 Hz, because they are the most common and correlate with environmental exposure (eg. power lines) [Tomitsch, Dechant 2015]. The molecular mechanism of action of the electromagnetic field to the cells and the selectivity induced effects is not fully understood, and required further studies. There is a few pathways on which the EMF may affect the viability and proliferation of cells. One of them is the induction of apoptosis, the programmed cell death that is dependent on oxygen free radicals, which level rises as a result of an electromagnetic field influence. Some studies have shown increased expression of HSP70, a marker of cellular stress response, as a result of stimulation by EMF [Lin et al. 2001; Takalov, Gutzeit 2004]. Also it is speculated that the efficiency of the electromagnetic field antitumor therapy is a result of change (increase) of the level of intracellular calcium ( $Ca^{2+}$ ) or specific signaling pathways [Wolf et al. 2005; Vijayalaxmi, Prihoda 2009; Simko 2007; Sadeghipour et al. 2012]. Exposure to EMF causes changes in cell membrane integrity allow the influx of  $Ca^{2+}$  into the cell through voltage-gated calcium channels T [Stratton et al. 2013; Saliev et al. 2014]. However, many tumor cell lines, among which there are the cell lines derived from breast cancer, MCF-7 and MDA-MB-231, are characterized by a unique overexpression of this type of ion channel which was not seen in nor-

mal cells [Taylor et al. 2008a; Capiod 2011; Taylor et al. 2008b; Ohkubo, Yamazaki 2012].

In summary, the observations made in this study are consistent with the idea of having the specific properties of the electromagnetic field, which can alter the functioning of biological systems. Thus, a selective mechanism for killing tumor cells opens the possibility of using a technology based on the electromagnetic field to the preferential destruction of breast cancer cells under clinical conditions.

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