Problems of Investigations in Sphere of Electromagnetic Fields Impact on Biological Objects

Anna V. Busygina, Maxim E. Komnatnov, *Student Member, IEEE*, Olga A. Matveyenko
Department of Television and Control
Tomsk State University of Control Systems and Radioelectronics, TUSUR
Tomsk, Russia

***Abstract* — Problems of the investigations in the sphere of electromagnetic fields (EMF) impact on biological objects (BO) in the TEM-cell have been considered. Researches on BO exposed to EMF (cell cultures, plants, worms, insects, animals) have been systematized. Features of devices for climatic (temperature, humidity, CO2 and nutrients) and electromagnetic exposure needed for research of specific BO have been described. The need for temperature control and video monitoring during the research on BO exposed to EMF has been justified. The results presented may be useful for development of research methods for investigation of the EMF impact on specific BO, as well as for the development of technical requirements in order to provide devices useful for such studies.**

Keywords — biological objects; electromagnetic fields; TEM-cell

I. INTRODUCTION

Nowadays, people are exposed to various technogenic factors, one of which is the electromagnetic radiation (EMR). As a result of technological development in the recent decades, the prevalence of this factor increases. It has been found that the EMR impacts molecular genetics, biochemical, and physiological processes in living organisms, so it is impossible to overestimate the importance of this factor for human [1]–[4].

Electromagnetic fields (EMF) are still widely used in medicine. For example, magnetic resonance imaging (MRI) is one of the safest methods of diagnostics. Pulsed magnetic fields are used to treat fractures, as they increase the rate of accretion of bone tissue [5]. However, there is an opinion that EMF has negative effects, for example, it increases the risk of neoplasia. At the same time, there are no definitive results showing the carcinogenic nature of EMR. The experimental evidence being not sufficient, the radiofrequency (RF) EMF has been classified as "possibly carcinogenic to humans" [6].

Trends in the development of modern electronic devices (increase of the signal frequency spectrum; growth of computational performance and etc.) cause the increase in the intensity of the human exposure to the EMF. People use devices with wireless communication systems (mobile communication, Bluetooth, Wi-Fi, 3G) in everyday life, thus, their impact on the body should be studied carefully.

The abovementioned highlights the need for a thorough study of the mechanisms of the biological effects of EMF [7], and, therefore, it is necessary to develop methods and devices for the study of EMF impact on the living tissue [8].

Purpose of this article is to highlight important topics in the area of electromagnetic fields impact on biological objects (BO), identify main issues of research and their possible solutions.

II. EXPOSURE DEVICE

A transversal electromagnetic mode (TEM) cell is one of the most common devices for research on the effect of EMF. This device was originally developed for the research and testing in the area of electromagnetic compatibility (EMC) [9]. The TEM-cell consists of the central conductor and three volumetric parts of rectangular cross-section, two of which have a linear expansion of the cross sections in the shape of a pyramidal horn, and the third part is shaped as a cube with a regular cross section along the cell, in which the object of the study is placed (Fig. 1). EMF excited by the high-frequency generator inside the TEM-cell affects the equipment under test (EUT).

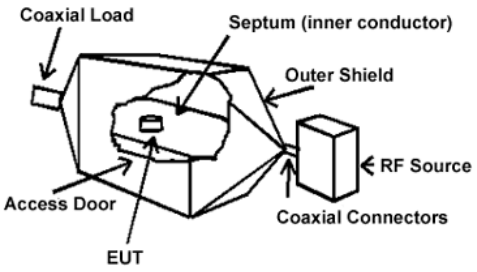

Fig. 1. Standard construction of the TEM-cell

Nowadays, the construction of the TEM-cell is being improved, which allows the researchers to conduct a wider range of tests on interference immunity and interference emission of various radio-electronic devices, and also to conduct the research in biophysics and biomedicine.

III. BIOLOGICAL OBJECTS AND RESEARCH FACTORS

As various BO are exposed to EMF during the experiments, the establishment of specific conditions typical for a particular object is required. Special design solutions are also required in order to place a BO inside the TEM-cell and ensure its correct positioning and fixation.

A. Cell cultures

To investigate the EMF impact on living tissues, the human cells were used (promyelocytes [3], fibroblasts [7], [10], leucocytes [11], amnion epithelial cells [12], [13], [14]) and animal cells as well (rat myocytes, guinea-pig myocytes [15], neuronal cells [16]).

Cells can be cultured as monolayers [7], [13] using the artificial substrate in a Petri dish, or they can be grown in suspension [7]. In paper [7] the uniformity of electromagnetic energy absorption was provided for the area of the monolayer less than 50 cm^2 and the volume of the suspension less than 10 ml. The cell monolayer was divided into several Petri dishes, which were placed in the TEM-cell, and the suspension was also divided into several dishes. To investigate these objects, a special system of holders has been developed in order to place the Petri dishes inside the TEM-cell (Fig. 2).

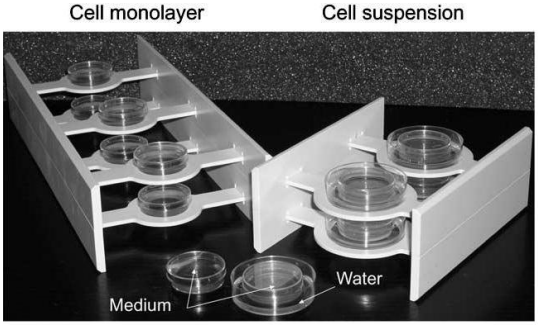

Fig. 2. Petri dish holders for cell monolayer and cell suspension [7]

Linz *et al.* [15] used round acrylic chamber (inner diameter 10 mm, height 6 mm) for experiments on the effects of radio frequency fields (RF) on the membrane potential; action potential and on the work of the L-type calcium channels of ventricular myocytes in suspension (total volume of 200 ml). It was found that the RF fields do not affect the resting potential and the action potential of the cell membranes. However, the authors note that other types of Ca^{2+} channels, such as N-type, which can be found in neurons only, might be susceptible to RF fields.

Research is conducted to identify the level of DNA damage caused by the EMF exposure. Thus, in paper [10] the human fibroblasts and rat granulosa cells were used as biological material, they were placed in the Petri dish with a diameter of 35 mm. Six Petri dishes were exposed to the EMF at the same time. The experimental results showed that RF EMF exposure causes single- and double-strand DNA breaks. The researchers emphasize that DNA damage can not be caused by thermal effects.

Several researches are conducted to investigate the impact of EMR on the protein structure. For example, [17] during the analysis of the impact of pulsed electric field and temperature on the structure of chicken egg lysozyme, Zhao and Yang have found changes in the conformation of the active site of the protein, inhibiting the binding of substrate to the protein and destabilizing the protein structure.

B. Plants

Much attention is given to research on the impact of EMF on plants, as they are an essential element of the Earth's ecosystem. For example, plant *Lemna minor* is the object of study in [1]. Plants of experimental and control groups were grown in the laboratory in a growth chamber and then were moved into the TEM-cell for the experiment, afterwards they were returned to the starting position. Such movements are stressful for the plants and affect the reliability of the results of the experiment, so it is necessary to exclude the movement of the objects during the experiments. During the investigation, the negative impact of electromagnetic fields on growth of the *Lemna minor* was revealed (reduction in the number of leaves in the second week of the experiment comparing to the control plants).

C. Worms

In the paper [2] devoted to the impact of continuous microwave fields on the nematode *Caenorhabditis elegans*, De Pomerai *et al.* have found that the heat shock proteins are generated, and the nematodes are growing in the accelerating rates.

To track a possible temperature rise associated with microwave radiation, the small volumes (0.2 ml) of concentrated suspension with nutrient medium and worms (in ratio of 1 to 2) were exposed to the radiation. In order to check the results of the experiment, identical, but shielded, control samples were placed nearby the experimental ones.

D. Animals

Researches on the impact of EMF on the whole body are carried out using laboratory animals, and this represents new challenges to developers. The animal is placed in the TEM-cell, so the field will be greatly distorted due to inhomogeneity. In paper [18] it has been shown that theoretical modeling can be used to determine the field distortion in the TEM-cell caused by the presence of structures with losses. It has also been shown that the distribution of field and the power absorbed in the structure depends on the size and the position of an object in a TEM-cell. Images obtained from magnetic resonance or computed tomography can be used as a realistic three-dimensional model of an animal.

For example, experiments on animals have been implemented in the paper of Gatta *et al.* [19], who investigated the impact of EMF on the proliferation of mouse spleen lymphocytes. During the exposure the mice were placed into the individual transparent boxes with holes for air circulation. To increase the uniformity of the field exposure, the mice inside the cell were rotated clockwise all the time. The control group of mice was also placed in the TEM-cell (under similar conditions), but not exposed to the electromagnetic field. The

results of this study did not show statistically significant differences in the rate of lymphocyte proliferation neither in the experimental, nor in the control groups exposed to the EMF of various power.

In paper [20] the system which can place up to 12 adult mice has been demonstrated, the length of the TEM-cell in this case is 120 cm. The mice were placed into the in groups of two. To ensure the proper location in the cell, the adult mice were placed inside the cylindrical devices fitted with small holes for evaporation, which were made of materials that do not affect the parameters of exposure, newborn mice were wrapped in the gauze and were placed in the polystyrene blocks.

E. Insects

Panagopoulos, Karabarbounis and Margaritis [21] investigated the impact of the EMR in the near field antenna of a cell phone on the fertility of the *Drosoplila melanogaster*. Insects were placed in a glass flask in groups of 10. Investigations have been carried out by theopen method (using the unshielded construction), which provided a controlled impact on the object. In the future, in order to achieve accurate dosimetry, systems of shielded exposure should be used. The modulated RF radiation reduces reproductive ability of insects to 50%-60%, while not modulated RF radiation reduces it to 15%-20% only.

IV. PROLONGED STUDIES

According to [12], the scale of the biological impact of EMF exposure depends on the exposure time, and the maximum effect is achieved by prolonged exposure. During the researches devoted to the identification of the biological effects of prolonged exposure of BO to EMF, it is necessary to maintain optimal environmental conditions in the TEM-cell.

Maintenance of the conditions needed for the BO includes some basic aspects:

- *Breathing.* Particular level of oxygen determines vital functions of BO, so it is important to ensure unhindered access of oxygen to the BO.
- *Nutrients.* During the prolonged studies, BO need appropriate nutrients that provide their normal functioning. At the same time, nutrients should be delivered to the object periodically, without removing the object from the exposure.
- *Temperature.* Temperature level comfortable for a BO will eliminate additional stress, which can affect the results.

For example, in the studies concerning fertility of insects, it is necessary to take into account factors that affect the oviposition (temperature, humidity, the amount of nutrients and population size). Studies [21] created the following conditions: temperature 25°C, 12 hours of light and darkness, humidity of 70%, nutrient medium was placed on the bottom of the flask.

During the studies of the impact of EMF on the body's cells, the climatic conditions should be maintained in an incubator: temperature of 37°C and certain atmospheric conditions (95% air / 5% CO_2) [3], [10], [11], [13], [22], [23]. In the papers of Diem *et al.* [10] and Merla *et al.* [16] the required level of humidity of 95% was maintained. In paper [2], for worms the temperature 24°C–25°C was maintained.

In the paper of Ardoino *et al.* [20] in order to supply air to the animals, the removable grid-wall was built into one side of the outer conductor of the TEM-cell.

The exposure system developed by Komnatnov and Gazizov [24] provided a tube through which nutrients are delivered directly to the object under exposure.

V. TEMPERATURE CONTROL

Previously, it was thought that the EMF has only thermal impact, but some studies have shown that there are non-thermal effects of the EMF exposure [3], [13]. Therefore, during the experiments on the EMF impact on living objects, special attention must be paid to temperature control.

- This will evaluate the role of temperature in the possible biological effects associated with EMF exposure [20]–[11], [25], [14].
- Maintenance of the physiological temperature level will eliminate the thermal effect, so it will provide a thorough examination of non-thermal effects.

Temperature measurements

In the study [2] the temperature of the suspension was measured after the EMF exposure: plates with samples were sequentially extracted from the incubator, the temperature was measured immediately (8 measurements per minute). Such measuring caused a decrease in the temperature of the subsequent samples comparing to the first ones.

During the experiment in the study [20] the temperature sensor was placed under the belly of the mouse for temperature control.

In [23] Kohler *et al.* used a temperature-sensitive fluorescent markers (rhodamin) for temperature control, which do not disturb cellular functions and can be used for visualization of the cellular and subcellular structures for a long period of time (up to several hours). Fluorescent lighting of the cells was performed using the light diode, which was connected to the microscope with the 1 mm quartz fiber. Besides, they used the fiber optic temperature probe for monitoring and evaluating changes in temperature inside the Petri dish, the probe was placed vertically in the cell suspension through the holes in the upper wall of the TEM-cell and the Petri dish lid [11]. The other experiments also used the temperature probe, it was introduced into the device through a hole (diameter from 5 mm to 10 mm) in the structure of the TEM-cell [15], [16].

In the experiments of Ticaud *et al.* [26] and Jarrige *et al.* [27] the temperature in the Petri dish was measured by means of the electrooptical sensor. In the study [27] the sensor can simultaneously measure the temperature and a component of the electric field in a continuous wave mode or in a pulsed mode.

Cooling and heating in exposing system

Paper [7] presents the cooling mechanism, based on the rapid exchange of air by fans, when the air flows through the air intake, located in the wall of the incubator.

In the study [20] adult mice were placed in the environment at a temperature of 20°C, while newborn mice require a temperature of about 30°C. The system of required temperature used in this paper consists of two outer metal jackets which are connected with the upper and lower cell plates. Jackets were filled with circulating water and connected with a thermo cryostat. This system allows cooling and heating of the internal environment of the cell in accordance with the requirements of the experiment.

VI. VISUAL CONTROL

Nowadays, constructions of TEM-cells are divided into two groups: providing the blind exposure or real time monitoring. In the blind exposure systems, the changes in the test object are measured after the EMF exposure, so it is quite difficult to track the changes in the BO. Visual control systems in real time allow to determine changes in the object exposed to the analyzed factors, avoiding extraction of the object [1], [3] and, therefore, providing continuous exposure, monitoring and evaluation of exposure.

Example of visual control was presented in the paper of Komnatnov [24]. Presented module of visual control provides the opportunity to observe real-time changes that occur in the object of research and to carry out recordings of the received data in the video format. A disadvantage of this module is that it does not have the possibility of multiple image magnification needed for experiments at the cellular level. Work in this direction is being continued.

Paper [15] also represents the exposure system with the visual control. The bottom of the experimental chamber (bottom consisted of a cover glass) in the device is located under the hole in the bottom of the plate. It allows monitoring of cells using an inverted microscope. The holes were covered with copper mesh to prevent irregularities in the device field.

In the study [23] Petri dishes containing a culture medium were placed on the microscope stage of an inverted microscope. A small hole was made in the center of the bottom panel of the TEM-cell to observe the samples via a microscope with a long lens.

VII. JOINT IMPACT OF THE CLIMATIC AND ELECTROMAGNETIC FACTORS

In a real life human exists in a heterogeneous environment, undergoing various factors, which together form complex interactions. It determines the necessity for a comprehensive study of exposure combinations of different factors (temperature, humidity, EMF) [12], [17], [25], [28].

As discussed in Section IV, it is necessary to maintain certain atmospheric conditions for normal physiological activity of BO. A large number of studies were carried out at 37°C, humidity 95%, it provides a purity effects of EMF exposure. The combined effects of various factors and their simultaneous impact remains unknown.

Nowadays, a device for the EMC tests ("Environmental shielded TEM chamber") is being developed. This camera allows carrying out joint tests on climatic and electromagnetic immunity and emissions of small radio electronic devices [29], [30]. Due to the combination of climatic and electromagnetic factors, its application to biomedical research is actual [8].

VIII. CONCLUSION

The impact of electromagnetic radiation on biological objects is still ambiguous. Various studies have shown contradictory results. It is probably explained by the experimental methods. Various BO require specific experimental conditions, which should be considered at the stage of planning, design of the exposing device, dosimetry calculations and methods for assessing the effect of EMF. The use of shielded systems provides controlled impact on the object, it improves the accuracy and reproducibility of the results. Video monitoring in real time with multiple zoom is also needed, it allows evaluation of prolonged exposure of the microscopic biological objects to EMF. The need for continuous research emphasizes the importance of maintaining the proper life conditions for BO during the experiment. Thus, there is a necessity to develop new exposure systems and standardized methods for experiments devoted to the EMF impact on living objects.

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