EFFECT OF SUBTOXIC DOSE, EXTREME LOW FREQUENCY (ELF) ELECTROMAGNETIC FIELD (EMF) TREATMENTS ON LIVER ENZYME CHANGES IN *IN VIVO* TURKEY MODEL EXPERIMENTS

Péter Hausinger and Krisztián Sepp, Andrea Csikós, Marianna Radács, Zsolt Molnár and Márta Gálfi

Institute of Applied Natural Science, Faculty of Education, University of Szeged Hungary, Department of Environmental Biology and Education, Juhász Gyula Faculty of Education, University of Szeged
e-mail: galfi.marta@szte.hu, molnar.zsolt@szte.hu

Abstract
Natural electromagnetic field background radiation in Earth is 20-30 μT, which is a condition in terrestrial evolution. Today, however, in the technosphere-determined environment, intermittent extreme low-frequency electromagnetic fields are predominant. In the present work, we aim to investigate this topic area by *in vivo* systematic study of subtoxic and chronic electromagnetic field exposures in a turkey model.

Introduction
Spontaneous electromagnetic radiation (20–30 μT) showed no significant change in the pre-social time interval of biological evolution provided by terrestrial conditions. Significant changes in natural electromagnetic background radiation in terrestrial habitat were begun in the 20th century with the extensive use of electrical devices. All this resulted in locally increased electromagnetic fields (EMF). A few publications on the study of the biological effects of EMF (radio frequency, microwaves) examine the question of how low frequency (0 and 300 Hz) electromagnetic radiation can affect living organisms, consequently humans [1, 2, 3, 4]. Evidence suggests that cell processes can be influences by EMF, it appears that EMF represent global stress. Changes in animal behaviour occur in response to a variety of electric field types [3]. The effects of noise and extreme low frequency (ELF) EMF are well documented on psychological mood and mental disorders. Many animal and human studies have reported various effects on the central nervous system and cognitive disorders from exposure to electromagnetic fields emitted by mobile phones [5, 6, 7, 8].

Aims
In the present work, we intend to investigate this topic area with *in vivo* model studies of subtoxic and chronic ELF EMF exposures. We place particular emphasis on a more in-depth study of subtoxic effects, with the aim of possibly proving a hypothesis that environmental exposure in the absence of a specific sensor may elicit more extensive response mechanisms.

Methods
Test animals
Adult (♀, certified) turkeys- *Meleagris gallopavo* (b.w.: 5000–5200 g) were included in our studies according to animal care and research protocols (5 animals per group). The turkeys were kept together in the experimental protocol, except for treatment time intervals. Prior to the start of the experiments, the turkeys were conditioned for 1 week. The animals were identified by unique numbering.

Extremely low frequency (ELF) electromagnetic field (EMF) exposures

The exposure was provided by a device capable of producing an intermittent ELF EMF (8 ms energy exposure - 2 ms energy-free pause). During the treatment, the cages were covered with a 200 cm × 80 cm "magnetic blanket". In operating mode, the parameters of the Hungarian electrical service system (U = 220 V, ν = 50 Hz) were provided. Turkeys were treated with ELF EMF exposure at ν = 50 Hz every 8 hours for 20 minutes for 3 weeks after a one-week conditioning period. The treatment protocol was followed by a 5-week regeneration phase, i.e., a treatment-free time zone. The technical safety of the treatments was controlled by the electromagnetic equipment: ME3951A low frequency (NF) analyser, Gigahertz Solutions, Germany, B = 10 μT checked: PCE-EMF823 electromagnetic field was performed with a radio tester (Tursdale Technicale Services Ltd, UK).

In vivo experimental model

The experimental protocol was 9 weeks in which we worked with 5 animals per group. In this time band, the first week after the conditioning period, ELF EMF (U = 230V, ν = 50Hz, B = 10 μT) treatment was performed for 3 weeks (in the treatment group), followed by an additional five weeks in the time band without treatment the evolution of data patterns of regeneration enzymes. Control groups designed for treatments: (AC) absolute control that was not treated; (C +) positive control for which the equipment was set in standby mode; (C-) negative control for which the machine was turned off mode; and (SC) stress control, in which the entire experimental protocol was performed but using an ELF EMF-free blanket. During the experiments, blood samples were taken every seven days (in sterile samples of heparin and citrate from the subclavian vein), toxicity enzyme parameters were determined.

Enzyme measurement

Toxicity parameters of the model animals participating in the experimental protocol were detected by Dialab methods (DIALAB, Austria). For toxicological monitoring, serum biochemical enzyme parameters of blood samples obtained from the subclavian vein: serum aspartate aminotransaminase (AST, EC 2.6.1.1) according to Remaley and Wilding, 1989 [9], serum alanine aminotransferase (ALT, EC 2.6.1.2) according to Matsuzawa et al., 1997 [10], and gamma-glutamyl transpeptidase (γGT: EC 2.3.2.2.) Were measured (3 technical replicates and at least 5 replicates). Data were evaluated with ANOVA.

Results

In our work, our results were related to the absolute control (AC) values, because there was no significant difference within the control system (AC, + C; -C; SC), only discrete modulation, so the results were presented in the average kinetic relations of the AC controls.

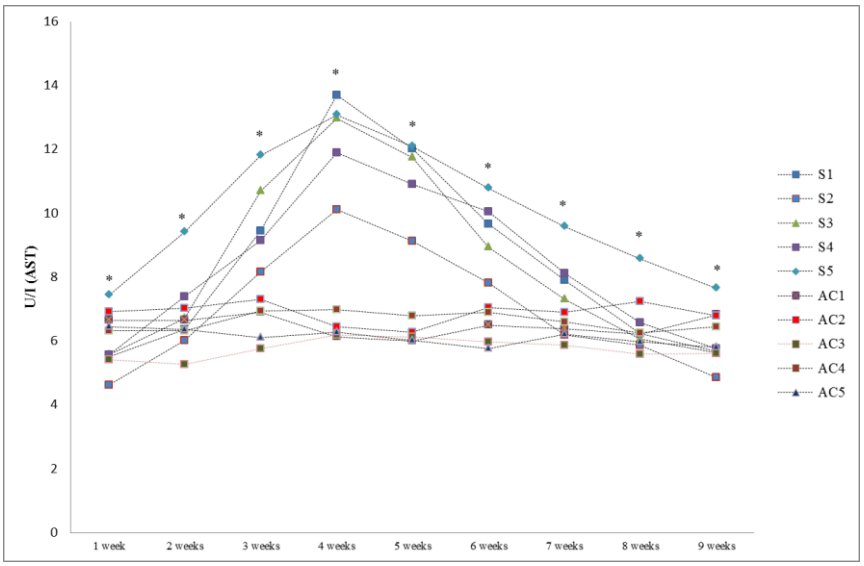

Figure 1. Changes in AST values as a result of ELF EMF treatments and changes in the AST enzyme during the regeneration phase (n=3, S: samples, AC: absolute controls, *: $p<0.05$ /related to ACs/)

It can be seen that the levels of individual AST enzymes were significantly shifted by ELF EMF treatments (2-4 weeks) after the conditioning period (1 week). However, the differences shown did not exceed the limits of normal enzyme levels (normal range: 1-40 U/L). AST enzyme levels returned to baseline during an additional 5 weeks of treatment-free, regenerative experimental period.

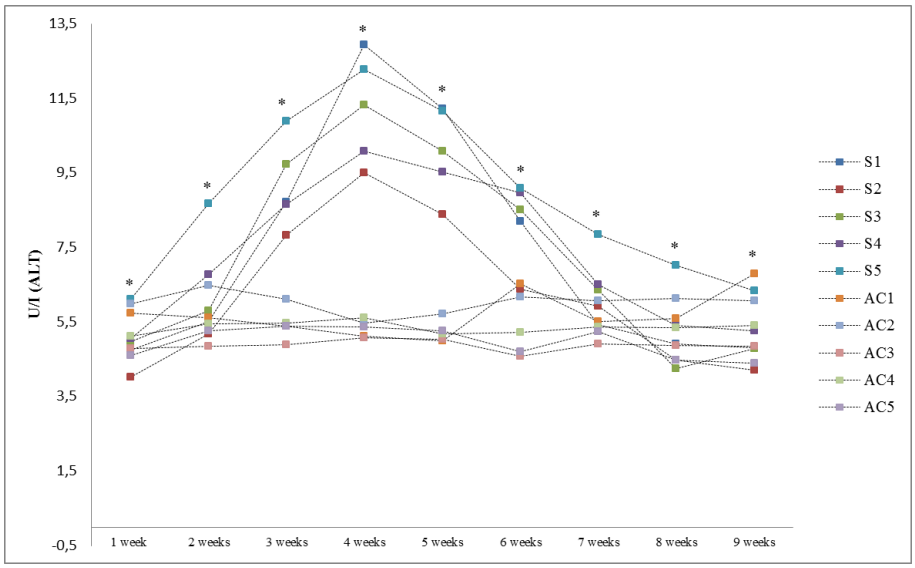

Figure 2. Changes in ALT values as a result of ELF EMF treatments and changes in the level of this enzyme during the regeneration phase (n=3, S: samples, AC: absolute controls, *: $p<0.05$ /related to ACs/)

It can be seen that the individual ALT kinetics were significantly modified by ELF EMF, although they remained within normal physiological range (1-50 U/L). Serum ALT enzyme levels returned to baseline during the treatment-free, regenerative experimental period.

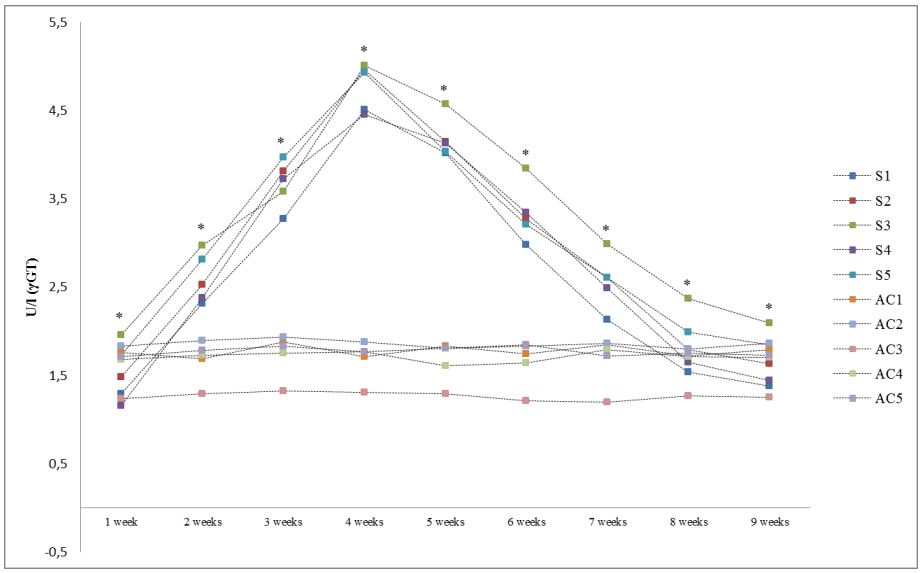

Figure 3. Changes in γGT enzyme levels as a result of ELF EMF treatments and changes in this enzyme level during the regeneration phase (n=3, S: samples, AC: absolute controls, *: $p<0.05$ /related to ACs/)

The figure shows that *in vivo* ELF EMF exposure significantly altered γGT enzyme levels, which returned to baseline levels during the untreated regenerative experimental period. The detected enzyme level changes varied within the physiological normal γGT (1-30U/L), enzyme level limits.

Discussion and conclusion

In our previous studies, we have described that the ELF EMF space altered noradrenaline-activated β-adreneregic receptor functions in the time dimension and dose we used. Because the aim was to study subtoxic doses, we could also describe that liver enzymes indicating toxicity did not leave the normal physiological range. In the present work, however, we specifically investigated whether it is possible to find ELF EMF effects following the individual liver enzyme kinetics within the range of physiological enzyme levels considered to be very broad-spectrum normal.

The presented kinetic curves clearly demonstrate that *in vivo* ELF EMF treatments had a direct effect on the synthesis results of enzymes signaling the functional function of the liver, pancreas, bile, and kidneys. As the ALT / AST ratio was below 1 in all cases, hepatotoxicity could be ruled out, so we performed our studies with truly subtoxic doses.

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