The effects of chlorobenzenes with the combination of extreme low electromagnetic field

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Abstract
Conditions of the evolution (phyisical, chemical and biological factors) determined the emergence and the survival of the earthly life. Natural conditions have been transformed by the presence of the society, change its contact networks. Accordingly, evolution takes place deterministically to the new condition.
There are some physical expositions which are not researched because of its low energy. Electromagnetic field is a very significant and it is caused by the electrical equipments.

Introduction
The natural and anthropogenic electromagnetic fields are parts of the environment, in which the living systems have been constantly exposed the effects of it. It is intriguing and likely important to investigate how the changes of natural background affect the adaptation. Electromagnetic field (EMF) effects on organisms have been rise, which was largely due to the technological advances [1, 2].
Trichlorobenzene (TCB) and hexachlorobenzene (HCB) are the derivatives of benzene. TCB is colorless liquid or solid, soluble in organic solvents, insoluble in water. HCB is a white, crystalline substance. TCB is used for insulating material, exchanger, component of synthetic oils and lubricants and insecticide [3]. HCB have been manufactured since the 1930's. Initially it was used as fungicide for the treatment of cereal. However, HCB measured in the nature originate from the accumulation of the HCB previously dropped [4, 5, 6].
Capacitors are for storing elementary charges, two conductive elements which are separated from each other with insulating material. In case of cells, there are great similarities with the capacitors' technical design. Biological systems can also be "capacitors", lipid bilayer is the insulator, plasma components are the leaders, and they are all sensitive for the changes of EMF [7].
It is known, that there is electric potential difference between the inner and outer surfaces of cell membrane, caused by the potassium, sodium and chloride ions.
The EMF effects can cause changes at about 2-5 eV. Radio and microwaves can modify the vibrational states of molecules, and also generate heat. Non-thermal effects can change the electrical properties of the cells with the modification of the membrane potential [8].
In our research methods we have created different types of cell models, and test them at standard conditions, which are essentials for the living organisms. Monolayer cell cultures will be able to show the consequences of EMF effects through function changes [9, 10].

In this present research, we aimed to study the effects of the extremely low intermittent EMF on cellular biological systems. Furthermore, we are looking for some standardized test method, through which we can follow the effects.
Because of this in this study, we would like to develop a method for study and follow the

effects of extremely low dose of intermittant electromagnetic fields and the subtoxic effects of chlorobenzenes (ClB).

Experimental

In vitro monolayer cell culture models from Wistar rats' pituitary and liver were made to investigate the electromagnetic exposure.

Control systems were set up. Samples for absolute control was not exposed to any test step (A(C)), n=10. We moved in experimental steps our stress control samples (S(C)), n=10. For investigate the electromagnetic field exposition, an ineffective head at negative control (-(K)) was used, n=10. In case of positive control an instrument with a capable of inducing exposition was used in zero position (+(C), n=10.

Changes in cell transformation can show the initiating action of DNA of cell cultures after chemical induction. Benz-c-acridine (1mg/mL, 24 h) was used as a certified dedifferentiated agent. We followed the DNA and the protein production of the cell cultures.

For test the function ability of the cell cultures we used proven differentiation crocin+retinoic acid (CRA) (1:1, 1 μg/mL, 5 days).

We test our standarded *in vitro* model system to search the effects of the extremely low dose intermittant electromagnetic energy. (50μT, υ = 60 Hz, for 5 days, /times of 6 hours for 20 minutes/)

In this, adenohypophysis (ADH), neurohypophysis (NH), hepatocyte (HEP) monolayer confluent cell cultures were treated (chemical /ClB: chlorobenzene mix / hexachlorobenzene: 2,4,6-trichlorobenzoene = 1:1/0,1μg/g protein; t=6 h, or physical EMF: 50μT, υ = 60 Hz, for 5 days, /times of 6 hours for 20 minutes, n=10).

During investigations we measured the ^{3}H-Thymidine incorporation and protein content of cell cultures with modified Lowry method and a Pierce BCA protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, IL, USA).

Results

Our results were described in two different graphs. It is shown in Figure 1 how the ^{3}H-Thymidine incorporation of the cell cultures was changed after physical and/or chemical expositions compared to absolute control. Protein production of the samples was shown in Figure 2.

There was not any notable difference between the control samples. For benz-c-acridine exposition there was a clear rise in DNA and protein production, which proves the operability of the model system.

Effects of ClB for DNA and protein production of various cell types did not show significant difference. We experienced similar to this, when the cell cultures were exposed to physical exposition.

There was negligible difference when we expose the cell cultures to at first, physical and then chemical effects. Conversely (chemical then physical), there was significant difference.

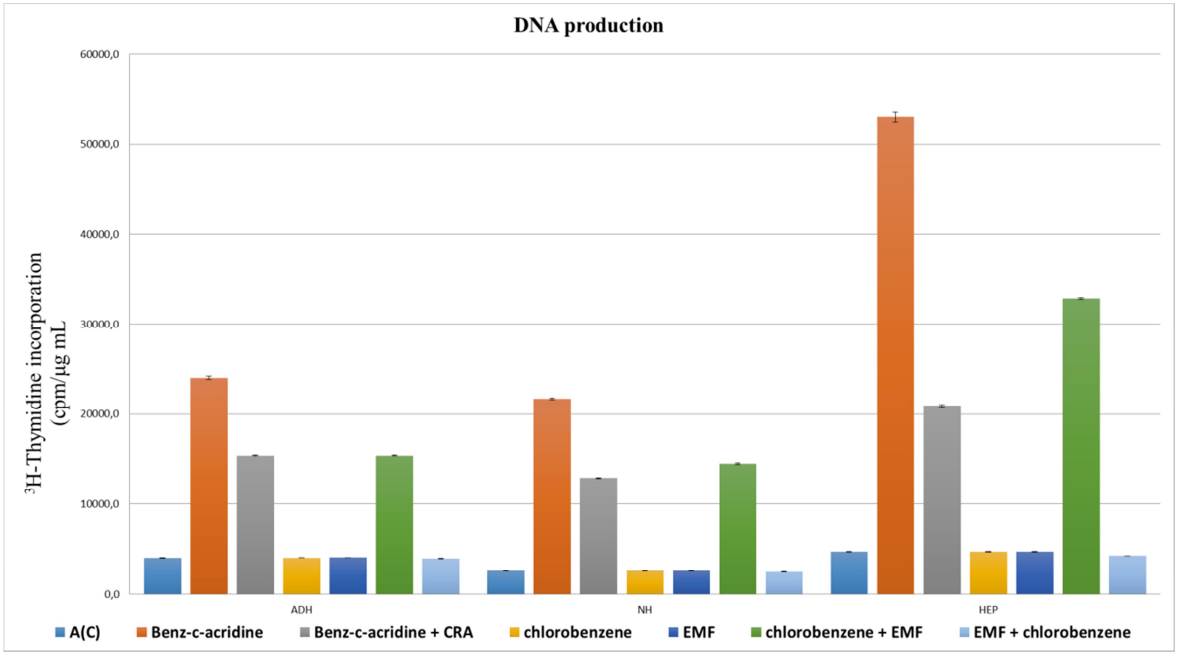

Figure 1
The effects of chlorobenzenes and/or electromagnetic field (EMF) on DNA production of the different types of cell cultures (n=10, means ±SEM)

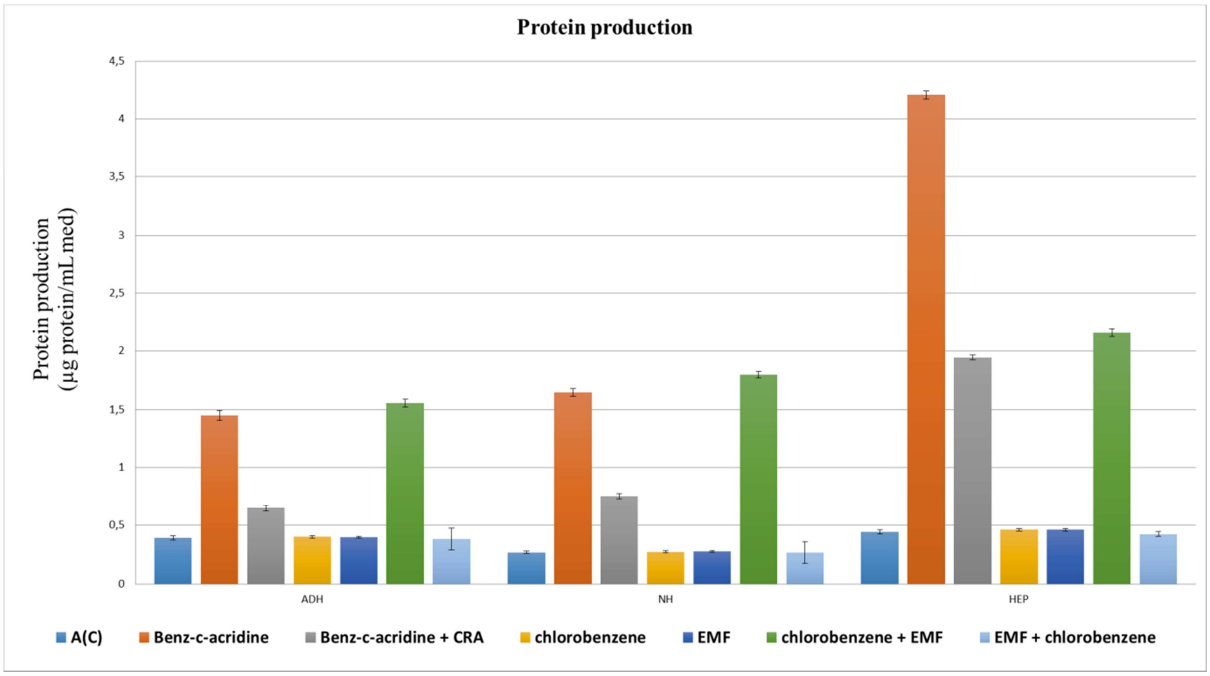

Figure 2
The effects of chlorobenzenes and/or electromagnetic field (EMF) on protein production of the different types of cell cultures (n=10, means ± SEM)

Conclusion

According to our results, we can state that the EMF exposure can cause large deviations on those cells, which was previously been exposed to an initiating effect by the ClB. The promoter effect of EMF can be considered. The physiological elements were modulated by these chronic, but extremely low doses of environmental loads (ClB with the combinations of EMF).

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