

Effects of long-term 50 Hz magnetic field exposure on the micro nucleated polychromatic erythrocyte and blood lymphocyte frequency and argyrophilic nucleolar organizer regions in lymphocytes of mice

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Submitted: 2008-07-22 *Accepted:* 2009-06-30 *Published online:* 2010-04-28

Key words: **electromagnetic fields; AgNORs, micro nucleated lymphocytes; polychromatic erythrocytes; mice**

Neuroendocrinol Lett 2010;31(2):208-214 PMID: 20424591 NEL310210A12 ©2010 Neuroendocrinology Letters • www.nel.edu

Abstract

OBJECTIVES: We aimed to investigate the effects of weak extremely low frequency electromagnetic fields (ELF-EMFs) on the nucleus size, the silver staining nucleolar organizer regions (AgNORs), the frequency of micro nucleated peripheral blood lymphocytes (MPBLs) and the micro nucleated polychromatic erythrocytes (MPCEs).

METHODS: One hundred and twenty Swiss albino mice were equally divided into 6 groups. The study groups were exposed to 1, 2, 3, 4 and 5 μ T 50 Hz-EMFs for 40 days. Micronucleus number (MN) per PBL was determined.

RESULTS: ELF-EMF exposure caused a nonlinear decline of nucleus area. A sharp drop occurred in AgNOR area of 1 μ T group, and following it gained an insignificantly higher level than that of the control group. The field did not change mean AgNOR numbers per nucleus of the groups. Relative AgNOR area had the highest level in 1 μ T-exposure group, and the level was quite similar to that of the 5 μ T-exposure group. The remaining groups had significantly lower values quite similar to that of the control level. The field exposure at any intensity did not affect significantly the frequency of either MPBLs or MPCEs. The number of MN per PBL in the 4 and 5 μ T-exposure groups were significantly higher than those of the lower intensity exposure groups. The males in 4 μ T-exposure group displayed the highest MN number per PBL, whereas values changed in a nonlinear manner.

CONCLUSIONS: The results of the present study suggest that $\leq 5 \mu$ T intensities of 50 Hz EMFs did not cause genotoxic effect on the mouse.

INTRODUCTION

Because the energy requirement of modern society mainly comes from electric power, it is difficult to find places in the living and working environment that are free from extremely low frequency electromagnetic fields (ELF-EMFs). The main ELF-EMF sources of everyday exposure are electrical power transmission and distribution lines, electrical substations, industrial appliances, ground currents of home wiring system and indoor appliances (Tenforde 1989). Residential exposures to distribution lines are usually under $0.5 \mu\text{T}$, but may be as high as $30 \mu\text{T}$ where the lines pass very close to common places in densely populated areas. Since typical indoor appliances, internal household wiring and industrial appliances generate stronger levels of ELF-EMFs, doubts have focused on possible deleterious effects of these fields on human health, because we have all been constantly exposed to such fields. Moreover, very close use of them to the body is the major problem with many indoor applications (Blank 1995).

ELF-EMFs with different field intensities, frequencies and waveforms have shown to cause alterations in various cell functions. So far, effects of ELF-EMFs on expression of cell surface markers, mRNA transcription, translation and the cell-cycle progression in both normal and tumor cells (Antonopoulos *et al.* 1995; Ruiz-Gómez *et al.* 2002), lymphocyte functions (Marino *et al.* 2001), mutation frequency (Fiorio *et al.* 1993), cell survival and expression levels of the apoptosis-related genes (Tian *et al.* 2002) have been investigated. Sometimes with contrasting results to date, no study has established unequivocally a causal relationship between EMFs and cancer.

There are several methods to test deleterious effects of physical and chemical toxicants on the DNA. Results of micronucleus induction test (MN test), size and number of silver staining nucleolar organizer regions (AgNORs) might give valuable information on the DNA damage. Khalil *et al.* (1993) determined clastogenic effects of long-term exposure of 50 Hz alternating EMFs by investigating chromosomal aberrations (CAs) in peripheral blood lymphocytes (PBLs) of high voltage workers. Single and double strand breaks (SSB and DSB) of DNA, MN frequency and sister chromatid exchange (SCE) have widely been used in the evaluation of genotoxic effects of ELF-EMFs (Fatigoni *et al.* 2005; Obe & Obe 2005).

A MN is formed when a damaged chromosome and its resultant fragments failed to be included in either of the two daughter nuclei. The genetic material left behind forms a "micro nucleus" in new generation cells. Since the MN frequency test is a good marker for DNA damage of the cells, MN induction test has largely been used as a sensitive genotoxicity assay under *in vivo* and *in vitro* conditions (Fenech *et al.* 2003).

Nucleolar organizer regions (NORs) are the sites in which ribosomal RNA genes are located. In silver

stained tissue sections, AgNORs are seen, as individual black spots or patches and they are mostly seen close proximity to the inner nuclear membrane in frequently dividing cells those which need large amounts of the ribosome. Chromosomal analysis of the mouse metaphase cells have showed that the cells had 10 AgNORs located on the 12, 15, 16, 18 and 19 chromosome pairs (Zaczek *et al.* 1994). Because these silver salts selectively stain actively transcribing NORs and rDNA, the researchers (Guler *et al.* 2005; Howat *et al.* 1989; Wachtler *et al.* 1986; Zatsepina *et al.* 1988) suggested that the size and number of AgNORs in tumor cells might show nucleolus activity and transcription level of the cell, and might be related to the cell proliferation. AgNORs have been reported to respond to environmental conditions, such as X-ray irradiation (Schwint *et al.* 1993), alcohol intake (Severgnini *et al.* 2002), smoking (Orrea *et al.* 2001), heat (Canet *et al.* 2001) and mechanical stresses (Hara *et al.* 2000) by significant increases in their number and size.

In this study, it was aimed to investigate the effects of 50 Hz fields with 1, 2, 3, 4 and $5 \mu\text{T}$ intensities on the cell nucleus area, some AgNOR parameters and frequency of the micro nucleated polychromatic erythrocytes (MNPEs) and peripheral blood lymphocytes (MPBLs).

MATERIAL AND METHODS

Animals and experimental design

In the study, 120 twenty-day-old Swiss albino mice were used. The animals were divided into 6 groups each having 20 animals (10 males and 10 females), as control (sham-exposure), 1, 2, 3, 4 and $5 \mu\text{T}$ ELF-EMF-exposure groups, after one-week habitation period. The animals were housed in plastic cages each containing 10 animals from the same sex, under a 12h light-12h dark schedule (light 06.00–18.00 hrs) at $21\text{--}23^\circ\text{C}$, and $50\pm 5\%$ relative humidity and fed with a commercial mouse food. Mice were on *ad libitum* water intake. The experimental group was continuously exposed to the field for 40 days. Control animals were handled and housed in identical conditions to that of the exposure groups, except field exposure (sham-exposure).

Ethics Committee of the Experimental Medicine Research and Application Center of Selçuk University approved all manipulations performed in this study.

Magnetic field exposure system

Homogenous magnetic field was created by using six solenoids similar to that reported by Mevissen *et al.* (1994). Each coil consisted of 300 turns of insulated copper wire that was single layered on a 32 cm diameter cylindrical plastic core. Plastic cages were located at the centers of the coils and each cage-coil system was settled in a laboratory in which ambient ELF-EMF intensity was less than 10 nT. Local temperature fluctuations in the cages were monitored periodically with

a digital multimeter (Maxcom MX 250TX) equipped with a high sensitivity PTC probe.

Each coil system was supplied with 50 Hz alternating current taken from local 220 V power network via a step-down transformer. MF intensities were measured in x, y, and z directions with a wide band, high sensitivity AC magnetometer (BBM-3D Model, Walker Scientific Inc., Rockdale Street, Worchester, Massachusetts 01606, USA).

Rms value was calculated with the given equation:

$$B = \sqrt{B_x^2 + B_y^2 + B_z^2}$$

Although ELF-EMF units are expressed as Tesla (T), amperes meter (A/m not generally used in the literature) or gauss (G; 1 G=0.1 T or 100 μT), T and G units are mostly preferred [10]. In this study, the intensity of the field flux was expressed as μT units.

Examination procedures and blood sample collection

At the termination of the exposure period, the mice were weighed and later euthanized with ketamine hydrochloride over dose (Ketanes®, Alke, Turkey). From each animal, 2 ml of cardiac blood sample was taken into heparinized (10 IU/ml blood) tubes. From each blood sample, four smears were prepared and fixed in glutaraldehyde-acetone for 3 minutes at -10 °C. Following fixation, the smears were rinsed in distilled water and then allowed to dry at room temperature for 30 minutes.

Staining of micronuclei and AgNORs

Two smears were stained for MN assay with modified May-Grünwald Giemsa stain (Suzuki *et al.* 2001). The incidence of MPCEs in 2000 polychromatic erythrocytes (PCEs) and lymphocytes (Mortazavi *et al.* 2005) per animal (in a 'blind' fashion on coded slides) were determined on high magnification digital images recorded with light microscope (Nikon Eclipse, E-400, equipped with Nikon DS-L1 Camera Control unit with DS-5M Camera Head).

In the remaining 2 smears, AgNORs were stained in the dark at 37 °C for 10–12 minutes, and then washed three times with distilled water (Korek *et al.* 1991), dehydrated in alcohol series, cleared in xylene and mounted.

Area of the nuclei and AgNORs was measured, and AgNORs were counted on the digital images. From the data, relative AgNOR area (AgNOR area/nucleus area ×100) was calculated. For the measurements, a digital image analyzing software (BS200 PRO 2005) was used. MN scoring in both PBL and PCE was done according to the criteria developed by previous researchers (Suzuki *et al.* 2001; Mortazavi *et al.* 2005).

Micro nucleated cell frequency was determined by investigating 2 000 polychromatic erythrocytes (PCEs) and 2 000 lymphocytes in each specimen and expressed as MN bearing cell number per 1 000 PBLs or PCEs. Mean MN number per PBL was also calculated from the data. A structure were concerned as MN: (I) clearly separated from main nuclei and located within the cytoplasm, (II) oval or round shape, (III) less than one fourth the diameter of the main nuclei, (IV) same textures and staining specifications as main nuclei.

Statistical analysis

The data obtained were analyzed using two-way analysis of variance. Since the data did not display normal distribution, non-parametric analyses were done. The significance of differences between control and exposed groups were analyzed using Kruskal Wallis test, and sex comparisons were done using χ² and Mann-Whitney U tests. The differences between the mean values of the groups with *p*<0.05 value were concerned as significant. In all analyses, SPSS 8.0 for Windows software was used.

RESULTS

Through the experiments, any clinical sign attributable to the field effect was not observed in the animals. Water and food consumption were in normal limits and the animals gained body weight.

Control group had the largest nucleus area. In general, the field exposure decreased nucleus area of PBLs, responses were nonlinear and individual differences were striking. The most pronounced decrease was in the 1 μT exposure group. Control, 3 and 5 μT exposure groups had larger nucleus area.

AgNORs were seen as black dots in the cell nucleus and mainly localized in close proximity to the nuclear membrane (Figures 1 and 2). The field treatment did not cause any change in the localization of the AgNORs. ELF-EMF exposure caused a nonlinear decline in the nucleus area. AgNOR area declined significantly with the 1 μT field exposure, then displayed a regular increase with the increase of field intensity and reached its peak level in 5 μT exposure group. The field effect did not affect mean AgNOR numbers per

Tab. 1. The nucleus and AgNOR parameters of the groups (Mean±SE).

Groups N=20	Nucleus area (μm ²)	AgNOR area (μm ²)	Relative AgNOR area (%)	AgNOR number per nucleus
Control	38.46±17.38 ^a	2.27±0.97 ^a	5.90±2.71 ^b	4.40±1.66 ^a
1 μT	15.02±8.64 ^d	1.10±0.89 ^c	7.36±10.97 ^a	4.45±2.76 ^a
2 μT	26.73±21.22 ^c	1.51±1.18 ^b	5.64±9.40 ^b	4.50±1.10 ^a
3 μT	33.76±11.99 ^{ab}	1.35±0.74 ^b	4.02±2.57 ^b	4.50±4.04 ^a
4 μT	28.73±8.88 ^{bc}	1.80±1.67 ^b	6.28±4.74 ^b	4.30±0.73 ^a
5 μT	32.86±8.74 ^{ab}	2.36±0.89 ^a	7.18±4.82 ^a	3.90±1.29 ^a

Means within a column followed by the different letter were significantly different.

Tab 2. The nucleus and AgNOR parameters of the females (Mean±SE).

Groups (n=10)	Nucleus area (μm ²)	AgNOR area (μm ²)	Relative AgNOR area (%)	AgNOR number per nucleus
Control	37.25±7.77 ^a	2.85±1.00 ^a	7.65±2.92 ^a	4.30±1.70 ^a
1 μT	16.42±10.83 ^c	0.75±0.75 ^c	4.56±8.96 ^b	3.40±1.65 ^a
2 μT	20.83±13.92 ^{ab}	1.31±0.59 ^b	6.32±9.57 ^a	4.40±1.17 ^a
3 μT	30.35±15.66 ^{ab}	1.48±0.95 ^b	4.89±3.24 ^b	5.60±5.58 ^a
4 μT	32.44±5.67 ^a	1.33±0.34 ^b	4.11±0.76 ^b	4.10±0.57 ^a
5 μT	34.41±12.82 ^a	2.28±1.02 ^a	6.64±5.88 ^a	3.80±2.58 ^a

Means within a column followed by the different letter were significantly different.

Tab 3. The nucleus and AgNOR parameters of the males (Mean±SE).

Groups (n=10)	Nucleus area (μm ²)	AgNOR area (μm ²)	Relative AgNOR area (%)	AgNOR number per nucleus
Control	39.68±23.96 ^a	1.69±0.51 ^b	4.26±1.33 ^b	4.50±1.72 ^a
1 μT	13.63±5.99 ^d	1.46±0.92 ^b	10.73±11.71 ^a	5.50±3.31 ^a
2 μT	32.64±26.07 ^b	1.70±1.59 ^b	5.21±9.61 ^{ab}	4.60±1.07 ^a
3 μT	37.17±5.68 ^a	1.23±0.46 ^b	3.32±1.21 ^c	3.40±0.84 ^a
4 μT	25.02±10.19 ^c	2.27±2.30 ^{ab}	9.09±6.10 ^a	4.50±0.85 ^a
5 μT	31.31±10.00 ^b	2.43±0.95 ^a	7.78±6.23 ^a	4.00±1.25 ^a

Means within a column followed by the different letter were significantly different.

Tab 4. Frequency of micro nucleated polychromatic erythrocytes (MPCEs) and peripheral blood lymphocytes of mice chronically (40 days) exposed to power frequency (50 Hz) magnetic fields (Mean±SE).

Group	Mean MN number per PBL			Mean MPBL frequency (MPBL/1000 lymphocytes)			Mean MPCE frequency (MPCE/1000 PCE)		
	♀+♂ (n=20)	♀ (n=10)	♂ (n=10)	♀+♂ (n=20)	♀ (n=10)	♂ (n=10)	♀+♂ (n=20)	♀ (n=10)	♂ (n=10)
Control	2.35±1.98 ^b	2.20±1.98 ^b	2.50±2.06 ^{bc}	7.01±1.21 ^a	6.98±1.01 ^a	7.04±1.19 ^a	0.95±0.57 ^a	0.93±0.17 ^a	0.97±0.10 ^a
1 μT	1.70±1.12 ^b	1.40±1.17 ^b	2.10±0.99 ^c	7.85±1.12 ^a	7.82±0.52 ^a	7.88±1.09 ^a	1.12±0.68 ^a	0.99±0.22 ^a	1.25±0.12 ^a
2 μT	1.80±1.50 ^b	1.70±1.70 ^b	1.90±1.37 ^c	7.58±1.33 ^a	7.18±1.11 ^a	7.98±1.33 ^a	1.11±0.41 ^a	1.09±0.32 ^a	1.13±0.14 ^a
3 μT	2.45±0.75 ^b	2.50±0.84 ^b	2.40±0.69 ^{bc}	8.05±1.37 ^a	7.98±1.07 ^a	8.15±1.37 ^a	1.37±0.66 ^a	1.18±0.23 ^a	1.56±0.13 ^a
4 μT	4.85±2.43 ^a	5.70±2.45 ^a	4.00±2.21 ^{ab}	8.10±1.47 ^a	8.09±1.47 ^a	8.11±1.41 ^a	1.42±0.57 ^a	1.36±0.22 ^a	1.48±0.16 ^a
5 μT	3.80±2.41 ^a	3.10±2.02 ^b	4.50±2.67 ^a	8.25±1.26 ^a	8.11±1.6 ^a	8.39±1.26 ^a	1.51±0.62 ^a	1.31±0.18 ^a	1.71±0.10 ^a

Means within a column followed by the different letter were significantly different.

nucleus of the groups. The proportion of the AgNOR area to the nucleus area of 1 and 5 μT groups were significantly higher than those of the remaining ones (Table 1). Area and number of AgNORs of both sexes in the control group were quite similar. Absolute and relative AgNOR areas of the experimental groups displayed significant sex dependent changes, whereas AgNOR number was not affected. MPBL and MPCE frequencies were the same for both sexes in the control and all of the experimental groups. AgNOR area significantly increased in the males of the 4 and 5 μT groups. Nevertheless, AgNOR area of the females responded to the field intensities in a similar manner to overall evaluation of both genders. No sexual difference was observed among the AgNOR numbers of the groups in response to the field effect (Tables 2 and 3).

MN were seen as small structures, with 1/3 size and similar staining characteristics to the cell nucleus (Figure 3). Field treatment did not change significantly frequencies of MPBLs or MPCEs. Controls and low-intensity field (4≤μT) exposure groups had lower MN number per lymphocyte, whereas the value significantly ($p<0.05$) increased in the 4 and 5 μT groups (Table 4).

The males in 4 μT group had the highest MN numbers, whereas the females displayed nonlinear changes.

The females in 1 μT group responded to the field exposure with a sharp and significant decline in the cell nucleus area, whereas it significantly increased in the females of both 4 and 5 μT groups. Nucleus area of the male animals changed nonlinearly in response to the field effect.

DISCUSSION

Household and office levels of ELF-EMF should vary from 0.01 to 1 μT, intermittent levels can reach more than 10 μT. As the aim of the present study was to investigate possible genotoxic effects of low intensity ELF-EMF on the mouse, the animals were exposed to 1–5 μT flux intensities, which were within range of the levels that one could encounter in the environment.

Intensive in vitro and in vivo experiments have also been carried out to clarify possible genotoxic effects of ELF-EMFs (Svedenstål *et al.* 1999; Ivancsits *et al.* 2003; Lai & Singh 1997). Although the possible genotoxic potential of magnetic fields is still unclear, studies indicated that occupational exposure to MF might increase

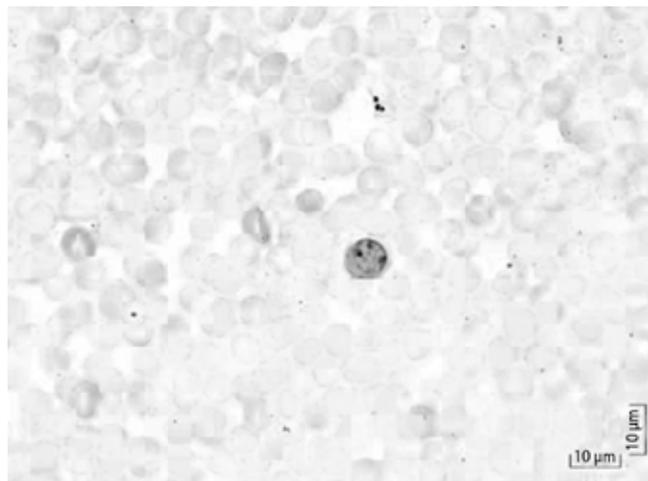


Fig. 1. AgNORs (black dots) in a PBL of sham-exposed animal. AgNOR stain, Bar: 10µm.

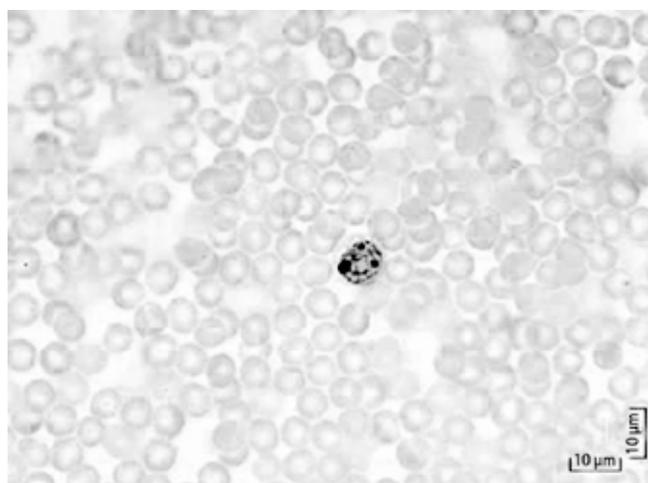


Fig. 2. AgNORs (black dots) in a PBL of 40 mG-exposed animal. AgNOR stain, Bar: 10µm.

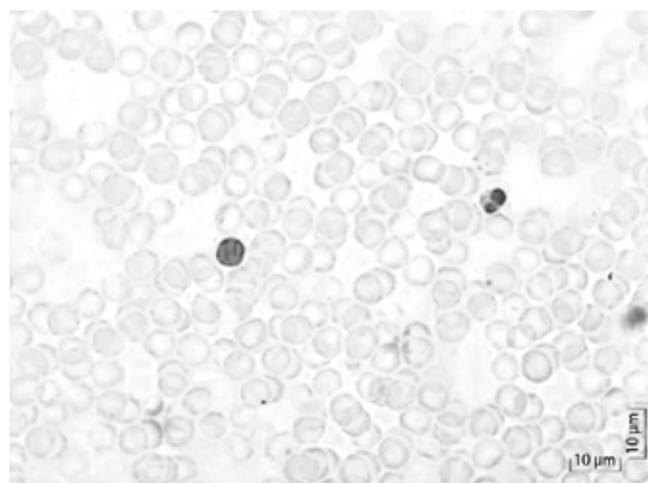


Fig. 3. MN in a PBL of 50 mG-exposed animal. MN stain, Bar: 10µm.

CAs in blood lymphocytes (Nordenson *et al.* 2001). Significant elevation in CA rates has been recorded in a number of workers in electrical occupations (Nordenson *et al.* 1984; Skyberg *et al.* 1993; Valjus *et al.* 1993). Adversely, Skyberg *et al.* (1993) concluded that there was no increase in cytogenetic damage among electric workers exposed to 60 Hz MF of 1.9 mT. These results raised considerable skepticism due to the absence of experimental evidence on the carcinogenicity of EMF exposure, and the absence of plausible biophysical mechanisms through which EMF could affect biological systems (Obe & Obe 2005). Lai & Singh (2004) have suggested that the damage caused by magnetic fields on DNA may depend on factors such as the mode of exposure, type of the cell studied, and the intensity and duration of exposure.

The existence of MN in a cell is concerned as evidence for chromatid and/or chromosome fragmentation, for damages in the mitotic spindle and evidence of numeric chromosomal aberrations. Since peripheral blood is much easier to obtain, PCEs (Abramsson-Zetterberg & Grawé 2001) and lymphocytes (Mortozavi *et al.* 2005) have widely been used to assess frequencies of MN in the genotoxicity studies, with increasing frequency. Base line frequency of MPCEs was given as 0.87 in per 1000 PCEs for peripheral blood (Çelik *et al.* 2005) and 0.4–6.5 per 1000 bone marrow PCEs in rats (Trosic *et al.* 2004). Abramsson-Zetterberg & Grawé (2001) found MPCE frequency as 0.96 per 1000 PCEs in adult females of controls, whereas the frequency was 1.27 in the males. The baseline MPCE frequencies in mice used in this study ranged between 0.93–0.97%, and males tended to display relatively higher MN frequency. Control animals showed higher MPBL base line levels (ranged 6.98–7.04 per 1000 PBL) when compared to those of the PCEs (Table 4). Nevertheless, the frequencies of control animals in this study were lower than reported by Mortazavi *et al.* (2005) who reported MPBL frequency as 11.33–12.0 for Bulb/c mouse lymphocytes. Inconsistent results on the effects of EMFs on MN frequency have been reported in previous studies. Abramsson-Zetterberg & Grawé (2001) found significant increases in MPCE and MNCE frequencies in 50 Hz 14 µT EMF exposed adult mice. Adversely, Udrouiu *et al.* (2006) did not detect any increase of MPCEs in adult mice exposed for 90 days to a 14 mT magnetic field; the similar results were obtained by Abramsson-Zetterberg & Grawé (2001), using an identical field, both in adult and newborn mice. Nevertheless, there has been an increase in the number of papers detecting genotoxic properties of power frequency (50 or 60 Hz) ELF magnetic fields alone with relatively high flux densities and various exposure periods, both in vivo and in vitro (Udrouiu *et al.* 2006). el Nahas & Oraby (1989) observed significant dose-response dependent MN increase in 50 Hz exposed mice somatic cells. The data of Simkó *et al.* (1998) indicated that EMF exposure at 0.8 and 1 mT (no increase at 0.1 and 0.5 mT) resulted in

a significant increase in MN transformed cells but not in non-transformed cells.

Paile *et al.* (1995) showed no significant increase in CA and MN frequency in 1 mT exposed cells and concluded that magnetic fields of less than 100 mT had no direct effects on chromosomes in humans. Similarly, in the present study, none of the applied field intensities (1–5 μ T) those were rather lower than effective level (100 mT) suggested by Paile *et al.* (1995), had no significant effect, either on the frequency of MPCEs or MPBL. In the present study, field exposure did not cause any change either in MPBL or MPCE frequencies at any of the applied intensities. Nevertheless, MN number per lymphocyte tended to increase at higher field (4 and 5 μ T) intensities. Females in the 4 μ T exposure group had the highest MN number per lymphocyte, whereas the value did not change significantly in the females of the remaining groups. Sexual and individual differences observed in MN number per lymphocyte might show that magnetic sensitivity was dependent on both gender and individual status of the animal.

Little is known on the AgNOR activity in the cells exposed to ELF-EMF. It has been suggested (Wachtler *et al.* 1986; Nordenson *et al.* 2001) that AgNOR numbers could be a useful sign of the protein synthesis activity of the cell types. The number and area of the AgNORs were found to be associated with proliferation activity of the cells (Zaczek *et al.* 1994; Skyberg *et al.* 1993). A close correlation has been reported between proportions of AgNOR area to nuclei areas of the cells. (Zaczek *et al.* 1994). There are limited data about the EMF influence on protein synthesis and cell proliferation. In a previous study (Kirschvink *et al.* 1992) a marked shift in DNA synthesis and phosphate intake have been observed in response to applied 40 μ T EMF at or close to ion resonance frequencies of Ca^{2+} , Mg^{2+} and K^{+} for 17 h (Nordenson *et al.* 1984; Yost & Liburdy 1992). In this study, cell nucleus area of the females in 1 μ T-exposure group responded to the field with a sharp decline, whereas the area significantly increased in the females of both 4 and 5 μ T-exposure groups. AgNOR area of the males significantly increased in the 4 and 50 μ T-exposure groups. Nevertheless, the females responded similarly to different field intensities as in overall evaluation. Any sexual difference was not observed between the AgNOR numbers of groups in response to the field effect. In this study, AgNOR area changed nonlinearly with the field strength. Interestingly, in low field density-exposure groups AgNOR area declined, whereas increased with the increase of the field density. Mean AgNOR numbers per nucleus of the groups were between 3.9–4.5. Field exposure was not effective on the AgNOR numbers. Although statistically not significant, relative AgNOR area was significantly higher in both 1 and 5 μ T-exposure groups. Our results are far from explaining the nonlinear nature of the investigated AgNOR parameters.

The results of the present study are quite far from giving evidence for the interference mechanism of EMFs with biological systems. Although, there is not a plausible mechanism in the literature, interaction of ELF-EMFs with DNA has been suggested to be quite complicated and apparently depends on many factors (Ivancsits *et al.* 2003). Moreover, Simkó *et al.* (1998) suggested that the EMFs had no initiating, but possibly a promoting capacity with respect to their suspected co carcinogenic competence. Cell membrane functions such as ion transport and ligand-receptor reaction have been proposed as possible targets for pulsed EMF (Tenforde & Kaune 1987; Cadossi *et al.* 1992). Two plausible biological mechanisms have been suggested to involve in the biological effect of ELF-EMFs. The first mechanism involves free radicals (Yaga *et al.* 1993), another one is based on EMR-induced alteration of cellular calcium ion homeostasis (Blackman 1990). Two other mechanisms, however, are in principle applicable at lower ELF fields, down to 10 μ T and possibly even lower. One of them interacts via magnetic crystals found in the tissues (Udroiu *et al.* 2006) the other model is that of ion parametric resonance (Rosen *et al.* 1998).

Based on these results, it is concluded that weak intensities ($\leq 5 \mu$ T) of 50 Hz EMFs had no genotoxic effect on the mouse. Since the results of the epidemiological studies implied that there might be a possible correlation between occupational ELF-EMF exposure and childhood leukemia, the efforts should be focused on the possible cellular mechanisms by taking into consideration of sexual and individual differences and keeping in mind the interaction with DNA is quite complicated.

ACKNOWLEDGEMENT

This study was funded by Scientific Research Application Center of Selcuk University (Project Number: 054-01-023).

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