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# Blood-forming system in rats after whole-body microwave exposure; reference to the lymphocytes

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**Abstract**

The influence of 2.45 GHz microwave (RF/MW) irradiation on blood-forming cells after whole-body irradiation of rats was investigated. The exposures were conducted with a field power density of 5–10 mW/cm$^2$, and whole-body average specific absorption rate (SAR) of 1–2 W/kg. Four experimental subgroups were created and irradiated 2, 8, 15 or 30 days, for 2 h a day, 7 days a week. Concurrent sham-exposed rats were also included in the study. The cell response was assessed by number and type of the bone marrow nuclear cells and peripheral blood white cells using standard laboratory methods. Significant decrease in lymphoblast count was obtained at 15 and 30th experimental day ($P < 0.05$), whereas other examined parameters did not significantly differed in comparison to the sham-exposed controls. The findings point out at stress response in blood-forming system in rats after selected microwave exposure, which could be considered rather as sign of adaptation than malfunction.


*Keywords:* Microwave; Rats; Bone marrow; Peripheral blood; Cells; Lymphocyte

## 1. Introduction

Biological effects have been observed at the non-thermal radiofrequency field intensities that do not produce measurable heating. At this time, however, these biological effects are not clear to have hazardous effects on biological matters. Evaluation of radiofrequency microwave (RF/MW) exposures was conducted primarily by military and industrial uses and, to some extent, broadcast exposures (Goldsmith, 1995). Nowadays, common concern on biological effects of microwaves grows because of increased general use of microwave generating systems. So far, many reported biological effects are simply indeterminate with respect to their significance to health (Elwood, 1999). Therefore, it is important that biological effects be understood, at least to the stage of their clinical significance, if any, so that health-hazard potential can be assessed.

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According to Repacholi's suggestion, the time course of effect should be investigated to determine if there are conditions under which effects disappear after rest, or if effects of individual exposures are additive, even after a rest period, or whether effects are permanent (Repacholi, 1998).

Of the epidemiological studies addressing possible links between RF exposure and excess of cancer, several positive findings were reported for leukemia and brain tumors. An increased risk of cancer in military personnel and higher rates of leukemia and lymphoma were reported by Szmigielski et al. (1988) and Szmigielski (1996). Increased incidence of childhood leukemia in the vicinity of radio towers has been reported by Maskarinec et al. (1994). In addition, a significant excess of leukemia cases among children and higher childhood leukemia mortality in relation to residential proximity to TV towers, and an excess of adult leukemias and lymphomas among the population with residence within 2–10 km of a transmitter have been reported (Hocking et al., 1996; Dolk et al., 1997a, 1997b). Other investigated health outcomes include spontaneous abortions, lenticular changes, neurological and sensitivity reactions, hematological or chromosome changes occurring in certain populations exposed to RF fields (Ouellet-Hellstron and Steward, 1993; Goldoni, 1990; Maes et al., 1993; Zotti-Martelli et al., 2000). Otherwise, there are well-conducted studies in military personnel exposed to RF, which are negative. Robinette et al. (1980) examined the health of US Naval personnel occupationally exposed to radiofrequency field below 1 mW/cm$^2$, and Groves et al. above 100 mW/cm$^2$. Their overall conclusion was that radar exposure had very little effect on mortality in this cohort of navy veterans (Robinette et al., 1980; Groves et al., 2002).

Therefore, the epidemiological evidence to date is inadequate for a comprehensive risk evaluation, and does not support a hypothesis of an association between exposure to radiofrequency fields and risk of cancer (Review of the Potential Health Risks of Radiofrequency Fields from Wireless Telecommunication Devices, 1999).

Besides, a number of biological effects observed in cells or animals during the RF field non-thermal level exposure have been reviewed recently by Heynick et al. (2003). It is recognized that non-thermal biological effects are measurable changes in biological systems that may or may not be associated with adverse health effects. Increased frequencies of chromosome exchanges and other cytogenetic effects (Manikowska-Czerska et al., 1985), a large scale structural rearrangement of DNA (Sakar et al., 1994; Lai and Singh, 1996), an excess of benign tumors of the adrenal medulla (Chou et al., 1992) and increase in the number of neoplastic colonies (Szmigielski et al., 1982) have been reported in rodents exposed to 2.45 GHz. Moreover, two-fold lymphoma frequency increase in transgenic mice due to 900 MHz exposure throughout 18 months was also referred (Repacholi et al., 1997).

It is known that activated or developing physiological systems are in general more sensitive to noxious stimuli than static ones. Because leucopoiesis is an ongoing process, there is continuous progression of cells from blasts to mature cells, which is balanced in the steady-state condition. Concerning bone marrow as the most proliferative tissue in the body; it is likely that hematopoietic system might reveal microwave effects, if any, even the subtle ones (Kreja et al., 1996). The 2450 MHz microwave induced changes in quantity and functionality in the circulating white blood cells and significant increase in the myeloid/erythroid ratio have been reported in exposed rabbits (McRee et al., 1980). Decrease in leukocyte count and percentage of granulocytes in peripheral blood of mice without disturbance in bone marrow cellularity after exposition to police radar frequencies has been reported by Rotkovska et al. (1993). In addition, the disorders in immunological parameters, both humoral and cellular, have been found in TV signal exposed workers. The increased IgG and IgA and decreased lymphocytes and T8 cells was observed by Moszczynski et al. (1999).

This study was a part of a more wide investigation designed to determine bio-indicators of 2.45 GHz RF/MW after whole-body rat exposure (Trosic et al., 1999, 2002; Trosic, 2001; Matausic-Pisl et al., 2000; Busljeta et al., 2001; Busljeta, 2002), and this paper describes cell response as assessed by determination of the bone marrow nuclear cells, in addition to the peripheral blood white cells according to a number and type, with particular reference to the lymphocytes.

## 2. Materials and methods

The experimental design has been described in detail elsewhere (Trosic et al., 2002). Male Wistar rats

(13-week-old, approximate body weight 350 g) were used in this study. A protocol approved by the Animal Care Committee (Institute for Medical Research and Occupational Health, Zagreb) was followed for handling and care of the animals. The animals had passed through a week accommodation period. Both sham-exposed control ($N$ = 24) and experimental animal group ($N$ = 40) were kept in steady-state microenvironment conditions (22 ± 1 °C), and receiving standard laboratory food and water ad libitum, with alternating 12 h light and dark cycles. A group of 40 rats was divided into four subgroups that were irradiated for 2, 8, 15 or 30 days. The sham-exposed animals ($n$ = 24) were also divided in four subgroups to be sacrificed on indicated days. During the irradiation procedure, animals were placed in individual plexiglas cages and exposed to RF/MW source (modified Micro-Chef Moulinex® generator, France) in the far field, at the distance of 1.4 m. Both, sham-exposed and exposed animal groups were kept in the same condition, except in the 2 h irradiation time daily for experimental animal group when RF/MW generator was switched on. The experiment has been carefully planed in order to satisfy basic general rules in the toxicology investigation and discrepancies, which appeared in number of treated and sham-exposed animal groups are allowed according to aforementioned in addition to the statistical principles. The animals were exposed to the 2.45 GHz RF/MW field 2 h a day, 7 days a week. The power density of the RF/MW field within each cage was measured by EM Radiation Monitor, type EMR-20 and 8.2 (Wandel and Golterman, USA). Mean total body specific absorption rates (SARs) were estimated according to a radiation dosimetry handbook (Durney et al., 1980). The selected power density range was 5–10 mW/cm$^2$, corresponding to approximate SAR of 1–2 W/kg for the middle-sized rat. The applied microwave power density has been reported not to affect the body temperature in rats (ICNIRP, 1998). Before and after exposure treatment, the rectal temperature was measured by ThermoScan thermometer (Braun GmbH, Germany) to avoid unpredicted thermal effects.

The blood sample was collected by cardiac puncture. White blood cell counts were measured by an automatic cell counter (Baker Seron System – 9210 CP, Biochem, USA). Bone marrow was isolated from a femur of each animal by the Mazur's method (Mazur, 1995). Absolute anuclear cell counts from each bone marrow sample have been determined using haemocytometer. Furthermore, the microscope slides of bone marrow smears were prepared and stained with May-Grünwald and Giemsa solutions. To perform the myelogram analysis, all slides were examined under 1000× magnification. Statistical analyses were conducted using the non-parametric Mann-Whitney and Kruskal-Wallis tests.

## 3. Results

The total number of nuclear cells from bone marrow of irradiated and sham-exposed groups of animals is presented in Fig. 1. Although there was slight decrease in nucleated cell number in 2.45 GHz microwave treated animals at the beginning of experiment, there were no significant differences between control and exposed animal groups during the 30-day lasted experiment.

Fig. 2 represents the absolute lymphoblast number obtained from bone marrow of irradiated rats and sham-exposed animal group. In comparison to the sham-exposed animal subgroups, the number of lymphoblast in irradiated animals was lower thorough the experiment. Significant decrease in lymphoblast number was obtained at 15 and 30 days of experiment ($P$ <0.05). However, the absolute number of lymphocyte in bone marrow did not significantly differ between treated and sham-exposed animal groups (Fig. 3).

Although there were obtained a slight drop in the blood leukocyte and lymphocyte number in irradiated animals when compared to the control ones, there were no significant difference between matched animal groups (Figs. 4 and 5).

## 4. Discussion

The results of our study show that 2.45 GHz whole-body irradiation during the 30-day treatment causes no serious alterations in white blood cells maturation and proliferation in bone marrow of rats. Except of the slight decrease in number of nucleated cells in microwave treated animals at the beginning of experiment, there were no significant differences between control and exposed animal groups. In Ragan's report (1983) of hematological and immunologic effects

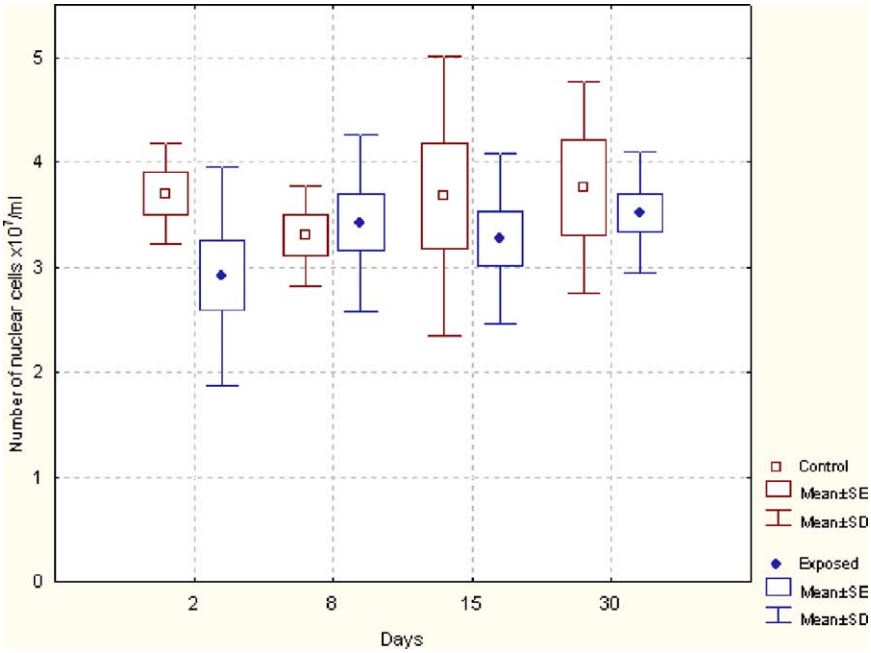


Fig. 1. The total number of nuclear cells from bone marrow of irradiated and sham-exposed groups of animals.

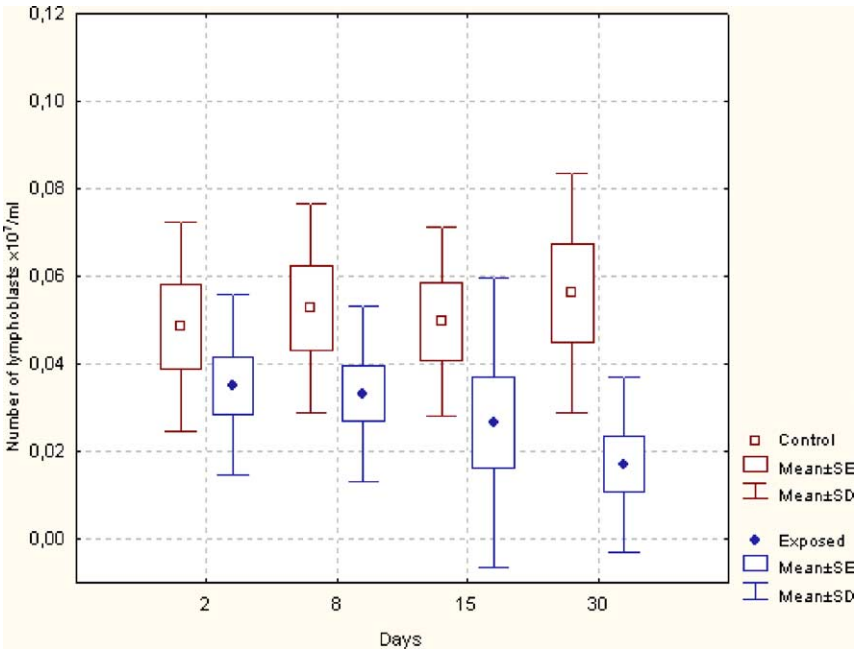


Fig. 2. The absolute number of lymphoblasts from bone marrow of irradiated and sham-exposed animal group.

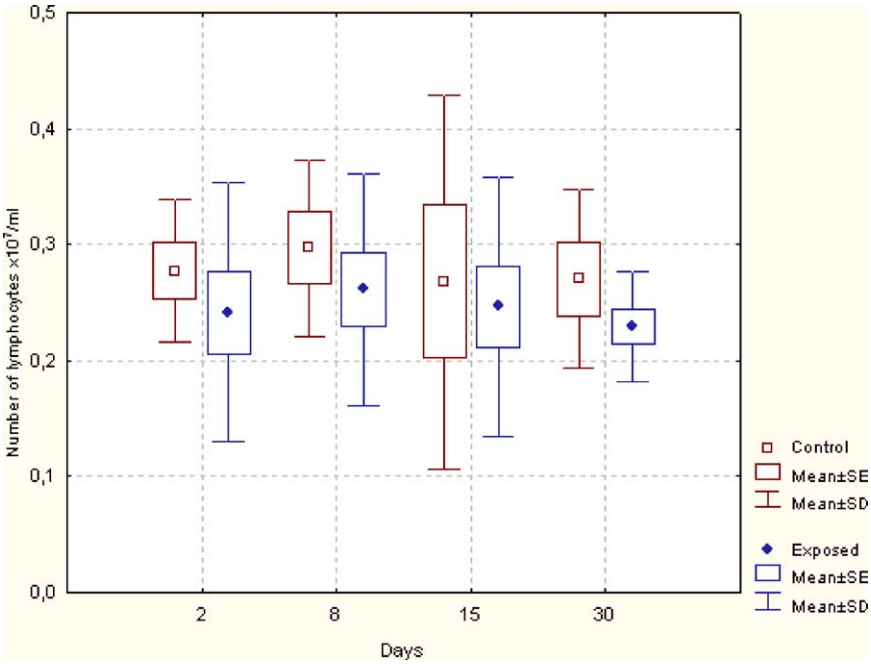


Fig. 3. The absolute number of lymphocytes from bone marrow of irradiated and sham-exposed animal groups.

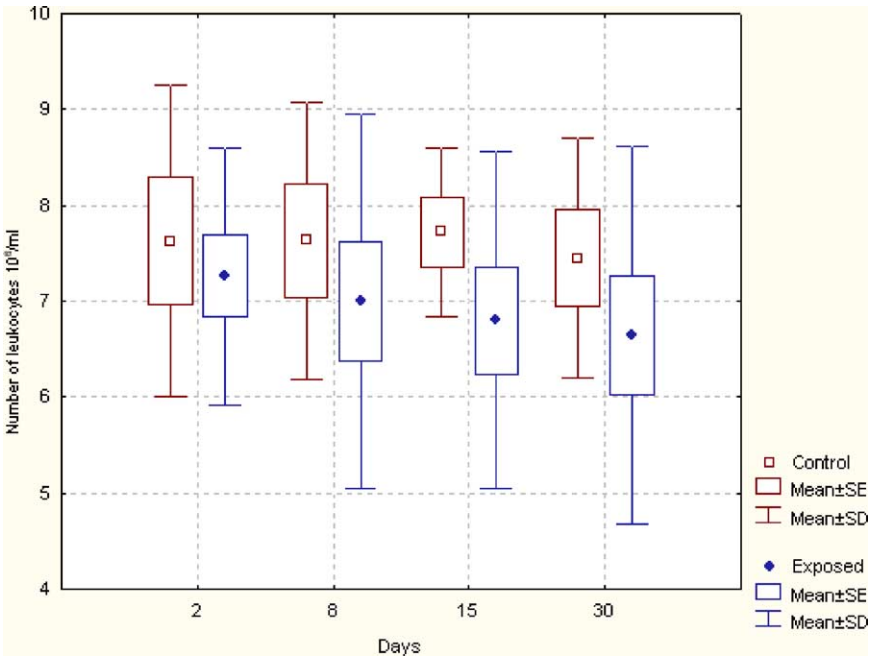


Fig. 4. The number of peripheral blood leukocytes in irradiated and sham-exposed animal groups.

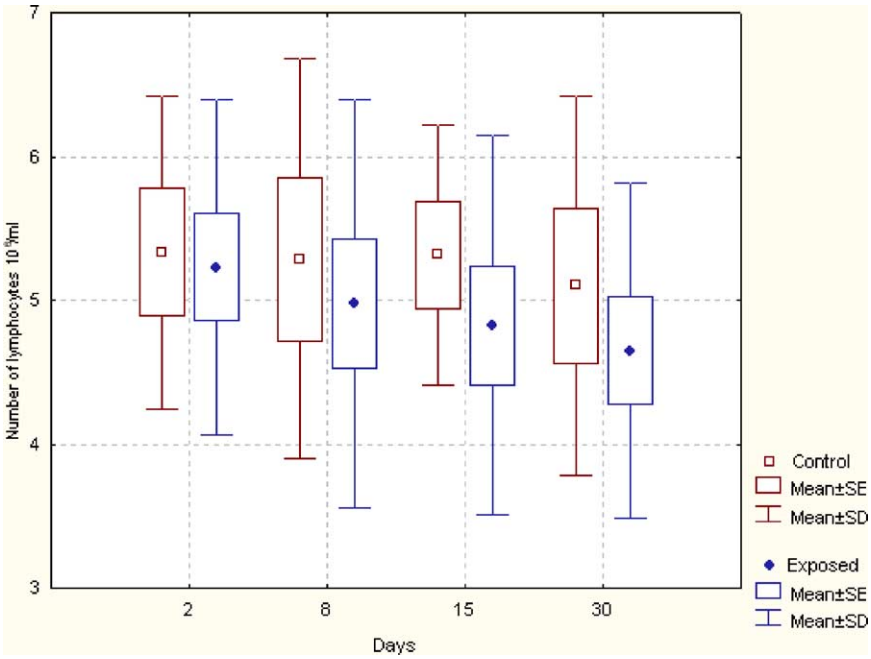


Fig. 5. The number of peripheral blood lymphocytes in irradiated and sham-exposed animal groups.

of microwaves in mice, bone marrow cellularity was significantly reduced in one of six groups exposed at 10 mW/cm$^2$, in contrast to increase in bone marrow cellularity in two studies exposed at 5 mW/cm$^2$. Results of these series of exposures of mice at SARs of 2.25 and 4.5 mW/g indicated no consistent effects on variables examined (Ragan et al., 1983). Slight, non-significant depression in rat bone marrow cellularity (Fig. 1) and the disturbance in lymphocyte maturation should be attributed to stress caused by initial and intermittent exposure to 2.45 GHz radiation, respectively. The number of lymphoblast in irradiated animals was lower thorough the experiment in comparison to the match sham-exposed rats, but significant decrease was obtained at 15 and 30 days of experiment ($P < 0.05$) (Fig. 2). It is possible that this non-thermal positive response could be due to so called sporadic positive responses. Reviews of assays used to detect DNA alterations have shown that such sporadic positive responses are indeed obtained from time to time (Verschaeve and Maes, 1998). The absolute number of lymphocyte in bone marrow did not significantly differ between treated and untreated animal groups (Fig. 3). These results are in agreement with no significant effect of 2450 MHz exposure intensities of 0, 2 and 10 mW/cm$^2$, i.e., SAR values of 0.44, and 2.2 mW/g, on the rat peripheral blood hematology (Galvin et al., 1982).

Considering the quantity of bone marrow nuclear cells, and the insight of the lymphocyte maturation process, the alteration neither in the absolute white blood cell count nor in the peripheral blood lymphocyte count was expected. Although a slight decrease in blood leukocyte and lymphocyte count in irradiated animals were observed there were no significant difference between matched, exposed and sham-control animal groups (Figs. 4 and 5). Collective evidence points to cell membrane receptors as the probable site of first tissue interactions with external radiofrequency microwave fields for many neurotransmitters, hormones, growth-regulating enzyme expression and cancer-promoting agents (Tenforde, 1997). In this view, theory by Fröhlich points out the electric dipole oscillations in membrane during irradiation, and consequently the instability of proteins that constitute the cell membrane (Fröhlich, 1968). Further step is the initiation of enzyme cascades that chemically couple cell surface RF signals to intracellular systems, including some that reach cell nuclei and

regulate processes of cell growth and division (Adey, 1999).

It could be concluded that selected RF/MW field under the applied conditions induced stress response in blood-forming system in rats after whole-body microwave exposure, but observed changes in the white blood and in the bone marrow cells are rather sign of adaptation than malfunction.

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