# Results of a Long-Term Low-Level Microwave Exposure of Rats

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***Abstract*—This paper summarizes the results of experimental research on biological effects induced by electromagnetic exposure to low-level microwaves. We exposed four-month-old Wistar albino rats during 21 months to two different microwave frequencies and exposure modes, 2 h a day, seven days a week. In order to assess possible biological effects of microwaves, we selected among others the following parameters: leucocytes, erythrocytes, monocytes, neutrophils, lymphocytes, hemoglobin, mean corpuscular hemoglobin concentration, and mortality rate. After three and eight months of exposure, we found a statistically significant difference of about 20% between the 970-MHz continuous wave group and sham-exposed group regarding the monocytes in both considered periods. After 14 and 18 months of exposure, we observed a significant increase in white blood cells and neutrophils of about 15% and 25%, respectively. Lymphocytes fell down after 18 months of exposure with about 15% compared to the sham-exposed group. No other statistically significant differences were found, except for minor changes with little biological significance. The most obvious effect we detected is the increase in mortality rate of the exposed groups with respect to the sham-exposed group after 21 months of exposure at the age of 25 months. This increase even increases when observing rats until the age of 28 months: mortality in exposed groups then reaches almost twice the value observed in the sham-exposed group.**

***Index Terms*—Biological effects of electromagnetic radiation, blood, continuous wave (CW) radar, military communications, rats.**

## I. Introduction

NEW technologies in personal communications lead to an increased exposure of the public to microwaves both in the private circle and in the office: cellular telephony, wireless communication devices, radars, etc. Today, millions of people are exposed to low-level microwaves, with the prospect that this will continue on a lifelong basis.

Workers, in particular military personnel in operation, are continuously exposed to fields from a plethora of sources.

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Besides, it is not uncommon to observe a high concentration of communication devices, mobile or ground-based implanted on a small compound in the immediate surrounding of personnel.

From the point of view of biological evolution, microwave radiation due to the proliferation of man-made sources constitutes a new environmental factor. The World Health Organization (WHO) and other international agencies have developed standards and guidelines, which are based on thermal and acute effects of microwaves. However, the debate on biological effects of long-term microwave exposure remains unsolved. Therefore, WHO stimulates further investigation about biological long-term effects of low-level microwave exposure [1], [2].

Biological effects in *in vivo* studies on blood cells have been found in animals exposed to radio frequencies, but mainly when a significant rise in temperature has been observed, while only a few studies demonstrated changes in blood cells at lower power density and specific absorption rate (SAR). It is of interest evaluating those hematological parameters that have been reported in prior studies as being altered by nonionizing electromagnetic fields in our exposure chamber specially designed for low-thermal exposure. Several studies have mentioned that thermal levels of RF result in increased levels of circulating neutrophils and decreased levels of lymphocytes [3], [4]. However, there are also a few studies revealing hematological effects at lower power densities. Baranski [5] exposed guinea pigs and rabbits to 3 GHz, pulsed and continuous waves (CWs), 35 $W/m^2$, 3 h/day for three months. Increases in absolute lymphocyte counts and no alterations in the granulocytes counts were observed. Goldoni [6] *et al.* reported that hematological examinations at a two-year interval in occupationally exposed workers showed a statistically significant decrease in thrombocytes and leucocytes. Ray *et al.* [7] exposed rats 3 h/day for 60 days at pulsed 7.5-GHz electromagnetic field (0.6-ms pulsewidth, average power level 0.6 $mW/cm^2$, SAR 0.03 W/kg); they found significant changes in hematological parameters. Budinscak [8] reported decreased erythrocytes, platelets, monocytes, and granulocytes cell count and an increased leucocytes and lymphocytes count in man at lower values of power density. Goldsmith [9] described changes in erythrocytes and leucocytes exposed to microwaves at an incident power density of 13 $mW/cm^2$. Matausic *et al.* [10] exposed rats to 2.45 GHz at an average power density of 10 $mW/cm^2$, 2 h/day, five days a week, during 30 days. Results revealed an insignificant increase in total erythrocytes. Total leucocyte count was significantly decreased for eight days of exposure, while relative lymphocyte count was significantly decreased from the first day. Trosic *et al.* [11] studied the

influence of 2.45-GHz microwave exposure, 5-10 $mW/cm^2$, whole-body average SAR 1-2 W/kg on the cell response assessed by the number and type of bone marrow nuclear cells and peripheral blood leucocytes. Significant decrease in lymphoblast count was observed, whereas other parameters did not alter significantly.

The results of studies concerning hematological effects of microwaves are often conflicting. The reason for such discrepancies is not always easy to identify and invites more well-conducted studies on laboratory animals [12].

Some long-term *in vivo* studies with rodents submitted to repeated high and low-level RF exposure indicate a positive relationship between exposure and carcinogenesis [13], [14]. Repacholi *et al.* [15] reported that long-term exposure of lymphoma-prone mice to one exposure level of 900-MHz RF radiation significantly increased the incidence of nonlymphoblastic lymphomas when compared to sham-irradiated animals. A replication study by Utteridge *et al.* [16] could not confirm this finding.

A few studies of a chronic exposure indicate a negative impact on overall health, and suggest the possibility that RF exposure may have epigenetic activity particularly at high levels [14], [17] . Nevertheless, there is no convincing evidence to show that RF exposure is genotoxic in animals [18].

In a study of Hruby *et al.* [19] on potential effects of 902-MHz global system for mobile communications (GSM)-type wireless communication signals, three groups of 100 female rats were exposed to three different SARs of 0.4 (low-dose group), 1.3 (mid-dose group), or 4.0 W/kg (high-dose group) for six months. Before, each group was given a single oral dose of 7.12-dimethylbenzanthracene (DMBA), to induce mammary tumors. All RF exposed groups had, at different times, significantly more palpable mammary gland tumor masses compared to sham exposure. There were statistically significant more animals with malignant neoplasms in the low- and high-dose group. In addition, the highest number of adenocarcinoma was found in the low-dose group. The authors concluded that the observed differences between the groups are not considered to give sufficient evidence for an effect of RF exposure on mammary tumor promotion or progression because of the fact that the cage control animals had significant more palpable tumor masses than the sham-exposed group.

Our paper reports on a long-term low-level microwave exposure of rats. In the literature review on 18 studies performed by Elder [20], there are very few long-term (21 months of daily exposure and more) studies reporting survival data in rats (whole body exposure). It is even harder to find mortality studies where rats have been exposed to frequencies beyond 2.45 GHz, as we observed the highest mortality in the 9.70-GHz exposed group. Liddle *et al.* [21] found an increased mortality in mice exposed to 2.45-GHz CW, 1 h a day, five days a week during 27 months at a whole body SAR of 6.8 W/kg. No effect on lifespan was observed in another population of mice that had been exposed to a SAR of 2 W/kg (1 h/day, five days a week, 31 months). Szmigielski *et al.* [22] reported an increased mortality both in mice exposed to a whole body SAR of 2–3 and 6-8 W/kg (2 h a day, six days a week, 10.5 months; 2.45 GHz). Utteridge *et al.* [16] found no statistically significant difference in mortality between sham-exposed groups and mice exposed to 898.4-MHz GSM exposure (1 h a day, five days a week, 24 months) at a whole body SAR of 0.25, 1, 2, and 4 W/kg. Adey *et al.* [23] reported no effect on survival of rats exposed to 836-MHz frequency modulation (2 h a day, four days a week, 24 months). In contrast with our study, the total exposure period was about half of which we considered. Besides, the continuity of the exposure was interrupted each time after four days, which allowed the exposed rats to compensate for an eventually occurred biological effect. Chagnaud *et al.* [24] found no effect on longevity of rats after exposure to 900 MHz. This study can hardly be considered when looking for long-term effects because rats were exposed 2 h a day for only two weeks. Toler *et al.* [25] and Frei *et al.* [26] exposed mice for 21 months (22 h/day) and 18 months (20 h/day), respectively. No decrease in survival was reported. It has to be mentioned that Toler submitted mice to a substantial lower frequency (435 MHz) than we did. In a long-term study of rats exposed to pulsed 2.45-GHz fields, Chou *et al.* [27] reported no statistical significant effect on survival rates; although survival was not affected, they found—if all primary malignancies were analyzed together—a statistically significant increase in tumors in the microwave exposed group relative to controls. The incidence of benign tumors did not differ.

## II. Specificities of This Study

The present study is a long-term low-level daily experimental investigation, exposing 124 rats for 21 months, i.e., about 640 days.

A total of 124 Wistar Hanover (Han) albino rats (*Rattus norvegicus*), separated into four groups of 31 rats, have been submitted to low-thermal microwave exposure. The experimental setup has been described in detail [28], [29]. Some behavioral aspects have been investigated during the course of the experiment and described previously [30].

The study mainly focused on effects on white blood cells (WBCs) (leucocytes), monocytes, erythrocytes, hemoglobin, and derivatives [among others mean corpuscular hemoglobin concentration (MCHC)]. A mortality study comparing the surviving rates between the groups has been performed, some preliminary results of which have been published [31].

At the end of the experiment, the surviving rats have been sacrificed and anatomopathological analysis on liver, lungs, kidneys, heart, thymus, bladder, spleen, brain tissue, testes, and gastrointestinal track is being undertaken. Presently, only a few rats have been examined.

## III. General Description of the Experimental Setup

The number of animals per group is limited. We have chosen the number of rats per group among other reasons basing ourselves on a certain statistical concern that a number of minimal 20 observations for each group of animals gives rise to a normal distribution [32]. Working with normal distributions facilitates statistical analysis afterwards. Against this background, we considered taking 31 rats for each group. There was also a practical and financial reason that has limited our population to this number of rats.

Three-month-old male Wistar albino rats, supplied by Charles River Laboratories, Horsham, PA, were first collectively acclimatized to the four spacious exposure units for

one month. In most studies, rats are kept separately during exposure, and space to move is limited.

Rats used in biomedical research are typically reared in small cages that lack key features of their natural environment. These conditions impose constraints on behavior, brain development, and stress [33]. Findings in animal psychology research learn that narrow housing causes an enormous stress response in the rat [34]. This stressor creates a supplementary variable, which is susceptible to mask secretion of certain corticosteroids [35], hormones released to cope with stressful situations. Restrained rats are stressed and the restraint stress affects the endocrine system such as pituitary-adrenocortical axis and sympathetic-adrenomedullary system. It has been shown that plasma levels of the adrenocorticotropic hormone (ACTH) can increase up to the fivefold of nonrestrained rats [36]. In this context, the effect of restraint induced stress was recently reported by Stagg *et al.* [37]. The observed levels of ACTH and corticosterone increased by nearly tenfold when conditioned rats were tube restrained without exposure. In unconditioned animals, the effect was even higher.

Stress is known to alter physiological homeostasis and distort experimental results [34], [35]. To our knowledge and according to the literature on *in vivo* RF related experiments, laboratory animals are exposed individually in the great majority of the studies. Most of them are caged in a small tube or in a waveguide where the space to move is very limited. Besides, rats are social animals, living together in rather smaller or bigger groups. Taking a rat out of his natural social environment is a source of supplementary stress. Therefore, we have chosen for an experimental design where the rats are collectively exposed in a self-constructed exposure unit. This spacious polyethylene box, adapted in a way to optimize the electromagnetic parameters, offers space for at least 40 freely moving rats. The entire exposure system is composed of four of those units, housing 124 rats all together.

Observing freely moving animals gives supplementary information on the influence of exposure on behavior and locomotor activity as an indicator of an effect on the central nervous system.

To establish a stable healthy colony of rats, it is best to start with a group of sexually immature individuals; or a single adult male with one or more females may be used, but that is not a proper solution because we intend to subject a sexually uniform group to microwave exposure. Due to the periodically variations in the female physiology, we prefer to work with only male rats.

Males brought up together from youth do not come into conflict. They are socially mature between 5–6 months [38].

During the entire experiment, a team of veterinarians followed up the health status of the animals.

Exposure system was designed for simultaneous exposure of the four groups, sham-exposed group included. This concurrence is important for statistical significance because it excludes differentiated influence of time component. The only variable is the exposure characteristic (frequency and mode, and sham).

In the literature, different kinds of exposure chambers have been described. D'Andrea *et al.* [39] constructed a chamber of plywood divided into identical halves by a partition covered with thin aluminium sheets (outside chamber dimensions $3.5 \times 3.5 \times 2.75$ m). Monopole antennas were mounted at the center of each ground plane. Other interior surfaces of the chambers were covered with absorber. Fourteen Plexiglas cages ($20 \times 10 \times 9.5$ cm) were mounted in a circular array 1.05 m from the monopole antenna of each chamber. The chambers were equipped with ventilating fans and 40-W houselights mounted on the wall opposite the antennas. The output of the magnetron was adjusted to obtain approximately 0.5 $\mathrm{mW/cm^2}$ at each rat position around the monopole antenna. The resulting SAR was 0.14 W/kg.

Another type of exposure system—designed by Tsurita *et al.* [40]—consisted of a small anechoic chamber, monopole antenna, and round turntable with eight tubes mounted circularly. The rats were confined in tubes. The head of the rats were positioned toward the central antenna, and each tube was ventilated from head to tail to decrease the stress of the rat by fresh air continuously flowing through the central duct into each tube. This sort of exposure chamber is more or less specifically designed for brain exposure of rats.

Bornhausen *et al.* [41] tested freely moving rats on behavioral changes after low-level exposure to 900 MHz. The field was radiated by a dish antenna atop the exposure chamber ($80\,\mathrm{cm} \times 80\,\mathrm{cm} \times 200\,\mathrm{cm}$). During exposure, the rats had access to food pellets and tap water. The radiated energy flux density was 0.1 $\mathrm{mW/cm^2}$. The SAR in the freely roaming animals was measured in models and ranged between 17.5–75 mW/kg.

In our experiment, all animals were housed in the same room of a conventional animal facility building with a 14/10 light/dark cycle with lights on from 07 a.m. to 09 p.m. at an ambient temperature of $23\ ^\circ\mathrm{C} \pm 1\ ^\circ\mathrm{C}$ and a relative humidity of $50\% \pm 5\%$. The ventilation rate of the room was 15 air volume changes per hour. Rats were given food and water *ad libitum*.

## IV. Choice of Exposure Schemes

Four different exposure schemes have been selected: 970 MHz CW, 970 MHz pulse-amplitude-modulated wave (PW), 9.70 GHz CW, and sham-exposure, respectively. The 970-MHz frequencies have been selected because of being representative of GSM cellular phone base-station exposure. The 9.70-GHz frequency has been chosen as being a radar frequency. A 9.70-GHz PW exposure has not been included because a generator that could deliver a peak power equal to ten times the mean power output of the 9.70-GHz CW was not available.

Four identical exposure units (Fig. 1), one for each exposure type, were used. Each unit consisted in a polyethylene cage ($1.11\ \mathrm{m} \times 0.60\ \mathrm{m} \times 0.71$ m), suitable for 31 freely moving rats, with an antenna on top. The height is such that the rats are in the far field of the antennas. The 970-MHz CW exposure unit is equipped with a Kathrein (GSM) antenna ($0.325\ \mathrm{m} \times 0.265\ \mathrm{m} \times 0.05$ m), characterized by a 9-dB gain and a 3-dB beamwidth in the horizontal and vertical planes of 65° and 70°, respectively. The second group of 31 rats is exposed to 970-MHz PW, with a pulse repetition frequency of 1 Hz, a duty cycle of 10%, an average power equal to the 970-MHz CW power, and an antenna identical to that of the first group. For the third group, exposed to 9.70-GHz CW, we used two $X$-band 16-dB horn identical antennas ($0.0725\ \mathrm{m} \times 0.05$ m), separated by 0.5 m in the plane of the

Fig. 1. Collective exposure system for freely moving rats.

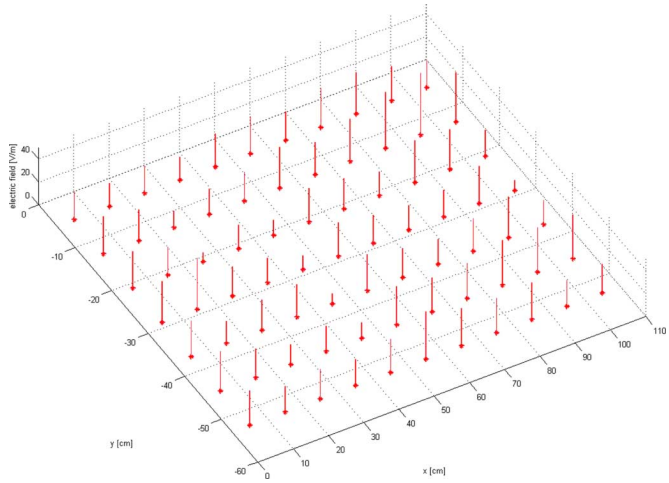


Fig. 2. Spatial representation of the electric field in the 970-MHz CW exposure unit.

cover of the unit to better distribute the radiated power over the entire section of the unit. Both horns have a 3-dB beamwidth of 28° and 30° in the $E$- and $H$-plane, respectively. The fourth group is sham exposed: rats were placed every day in their unit; however, they are not exposed to microwaves.

## V. Determination of Exposure Levels

The mean exposure level in absence of rats is, respectively, 2 $\mathrm{W/m^2}$ for 970-MHz exposures and 4.85 $\mathrm{W/m^2}$ for 9.70-GHz exposure. These are derived from the International Commission on Non Ionizing Radiation Protection (ICNIRP) guidelines [42] based on the human body. Considering that the ratio in length between a human being and a rat is equal to 10, we adapt these reference levels for general public exposure to the size of the rat by multiplying by 10 the two corner frequencies in the microwave range. Hence, the exposure of a rat at 970 MHz corresponds to a human exposure at 97 MHz. The basis ICNIRP reference value for man at 97 MHz is 2 $\mathrm{W/m^2}$. This value has been used for the rats at 970 MHz.

Similarly, 970-MHz exposure for man corresponds to 9.70-GHz exposure for rats. ICNIRP recommends a reference level for man equal to $f/200$ ($f$ is frequency), i.e., 970 MHz/200 equal to 4.85 $\mathrm{W/m^2}$ in the range 400 MHz-2 GHz, which corresponds to 4-20 GHz for rats. This level is chosen for the rats at 9.70 GHz. The corresponding whole-body SAR value is 0.08 W/kg, which is considered as low-thermal. It should be noted that we have not calculated the SAR inside of the animal. This would have required numerical calculation and a good evaluation of the boundary condition at the hair of the rat. Hence, we evaluate the SAR in the animal similarly to the way SAR is related to microwave exposure in human beings according to the ICNIRP guidelines.

The frequency-scaling application is well known: electromagnetic similitude is satisfied when a smaller size model is submitted to a correspondingly smaller wavelength, i.e., a correspondingly higher frequency [43]. This is strictly valid for no losses. Hence, SAR distributions will only be similar because there are obviously losses in living tissues.

In each exposure unit, the electrical vector is horizontal. The electric field was measured in nearly 80 sampling points inside of each unit (Fig. 2).

The measured mean power densities at 970 MHz and 9.70 GHz were 2.06 $\mathrm{W/m^2}$ and 3.20 $\mathrm{W/m^2}$, respectively. Hence, at 9.70-GHz CW, the rats have been submitted to a level lower than that calculated from ICNIRP. The overall electric field deviation is 30%, which can be considered as the best practical achievable distribution.

The experiment has been conducted under precise temperature control. During a trial exposure session, no change in rectal temperature between exposed and sham-exposed rats could be detected. Change in rectal temperature resulting from sham or microwave exposure was computed as post-exposure temperature minus pre-exposure temperature. The resting, grooming, and exploring by the animals indicated lack of thermal stress during exposure.

## VI. Blood Sampling

After testing several methods, blood was sampled by retro-orbital puncture [44]. Blood sampling was performed while rats were anaesthetized with a mixture of 8% sevoflurane (*Sevorane*, *Abbott*) and 92% medicinal oxygen (at a rate of 2 L/min) in a Plexiglas's induction box. The rat was taken out of the box as soon as the palpebral reflex disappeared. Between treatments, the box was cleaned with warm water and dried with a paper tissue. An Advia 120 hematology analyzer from Bayer Diagnostics carried out blood cell count and provided an automated analysis by counting and distinguishing different cell types. Blood sampling and analysis were performed in a blind manner: executers did not know to which group of rats the rat in question or the sample belonged. Blood was taken six times during the first 18 months of the exposure period, including one at the start of experiment, at an interval of about 3.5 months.

## VII. Statistical Analysis

Results are given as means ± standard deviations. After checking the normality of distributions (Kolmogorov–Smirnov test [45] and the homogeneity of variances (Levene's test [45], the data from the four groups of rats were analyzed using a one-way analysis of variance (ANOVA) [46] (Statistical Packages for Social Sciences (SPSS), version 15.0). In order to correct for multiple comparisons, we performed the Dunnett

test as a *post-hoc* test. We performed a multiple comparison test because this test is suitable for the simultaneous testing of hypotheses concerning the equality of three or more population means. In this case, the Dunnett test is appropriate [32]. It is specifically designed for situations where all groups are to be pitted against one reference group, i.e., the sham-exposed group [47]. Its goal is to identify groups whose means are significantly different from the mean of the sham-exposed group. As a *post-hoc* test, either the classic Dunnett or Dunnett T3 is used, depending on the equality of the variances of all our concerned groups. In the case of equal variances, the classic Dunnett test is used. On the contrary, when the variances are not assumed as equal, we run the Dunnett T3 test. A $p$ value $< 0.05$ was considered significant. Box plots used for visualizing differences display middle quartiles, the horizontal line in the box indicating the median and the whiskers depicting the range of the value for the considered parameter.

## VIII. Results

The statistically significant results we found during the duration of the experiment are mentioned in the following paragraph. It should be noted that no parameter exhibited statistically significant differences during *each* exposure period. Monocytes stopped showing significant differences between exposed and sham-exposed group beyond eight months of exposure. Leucocytes did not reveal statistically significant differences before a 14-month exposure period. After 14 months, the MCHC in exposed groups did not differ anymore from the sham-exposed group, while red blood cells (RBCs) and hemoglobin showed significances after three months of exposure. We did not find statistically significant differences in neutrophils during the first 14 months of exposure. Only after an exposure period of 18 months did we observe a statistically significant decrease in lymphocyte count. This variety of results confirms that results of studies concerning hematological effects of microwaves are often conflicting, as already mentioned in Section I.

For each dependent variable we proceed similarly.

### A. Monocytes

Fig. 3 shows the monocyte count in the different groups after a three-month exposure. The 23.5% increase in monocyte count in the 970-MHz CW group ($3.594 \pm 0.7989$) compared to the sham-exposed group ($2.919 \pm 0.8596$) is found to be statistically significant ($p = 0.006$), as is also the 21.1% increase in monocytes in the 9.70-GHz CW group ($p = 0.017$).

After eight months of exposure, the monocyte count is 20.6% higher in the 970-MHz CW group ($3.334 \pm 0.907$) than in the sham-exposed group ($2.765 \pm 0.837$), which is statistically significant ($p = 0.019$). The mean difference between the 9.70-GHz CW group ($3.237 \pm 0.736$) and the sham-exposed group, an increase of 17.1%, is nearly statistically significant ($p = 0.07$). After 18 months of exposure, we still observe a similar trend to increase in the exposed groups, but which does not reach statistical significance.

### B. Leucocytes (WBC)

Leucocytes count is expressed in $10^3$ cells per $\mu$L. Fig. 4 shows WBC count after 14-month exposure.

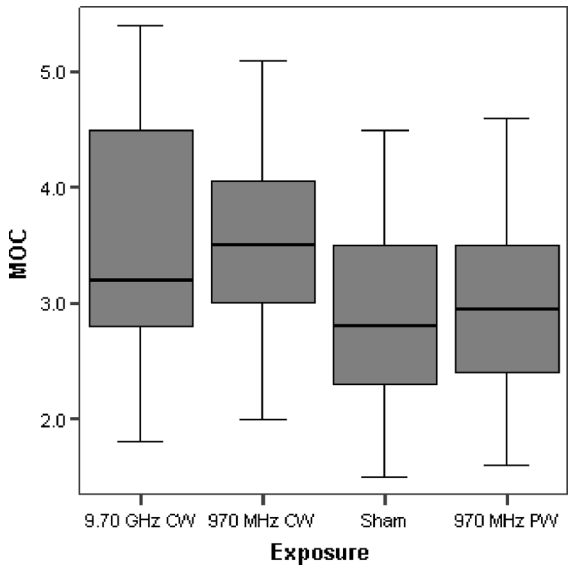


Fig. 3. Monocyte (MOC) count after three-month exposure.

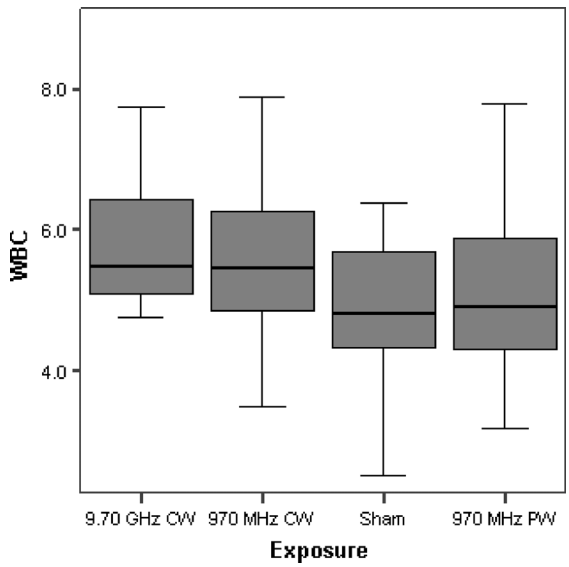


Fig. 4. WBC count after 14-month exposure.

WBC count in the 9.70-GHz CW group ($5.788 \pm 0.8724$) is 17.2% higher ($p = 0.012$) than in the sham-exposed group ($4.940 \pm 1.159$), while it is 14.1% higher in the 970-MHz CW group ($5.637 \pm 1.178$), ($p = 0.039$). Mean difference (0.697) between the 970-MHz PW ($5.138 \pm 1.0437$) group and sham-exposed group is not statistically significant ($p = 0.830$). WBC count in the 970-MHz PW group is 4.0% higher than in the sham-exposed group.

After an 18-month exposure, we find a statistically significant difference ($p = 0.002$) between the WBC count in the 970-MHz CW group ($6.939 \pm 2.171$) compared to the sham-exposed group ($5.192 \pm 1.195$). The increase is 33.6%. WBC count is 21.0% higher in the 970-MHz PW group ($6.283 \pm 1.425$) than in the sham-exposed group. This is nearly statistically significant ($p = 0.055$). Mean difference of 0.399 between the 9.70-GHz CW ($5.591 \pm 1.769$) group and the sham-exposed group is not statistically significant ($p = 0.712$). WBC count in the 9.70-GHz CW group is 7.7% higher than in the sham-exposed group.

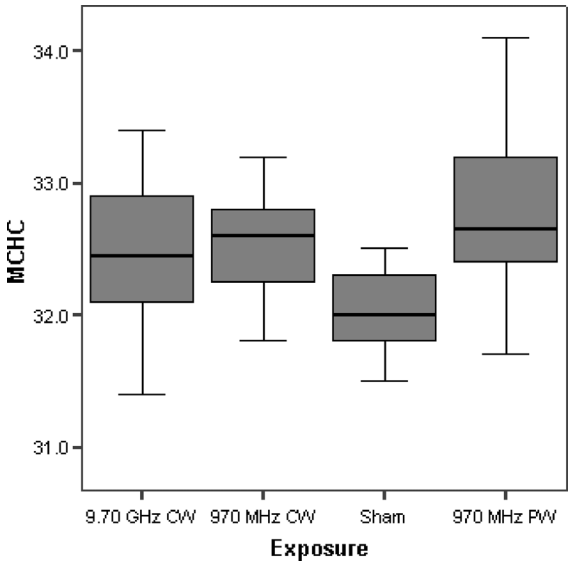


Fig. 5. MCHC after three-month exposure.

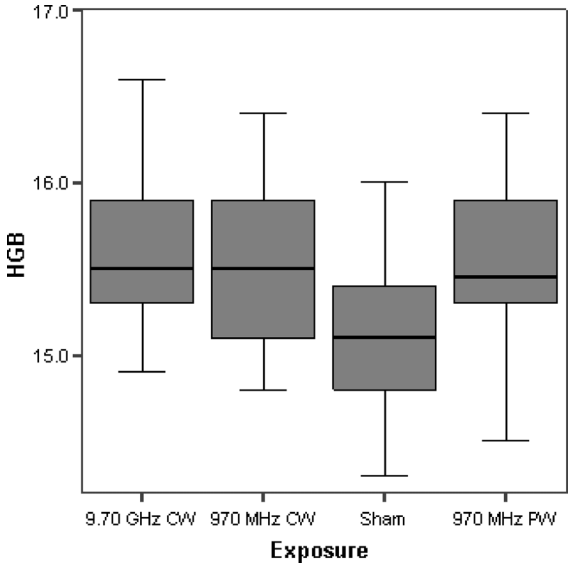


Fig. 6. Hemoglobin concentration after three-month exposure.

### *C. MCHC*

Fig. 5 shows MCHC, expressed in g/dL, in the four groups of rats after a three-month exposure. One observes an increase in all exposed groups with respect to the sham-exposed group. The increase is of the order of 2%. Mean difference between the sham-exposed group and the three exposed groups is statistically significant with a $p$ value of 0.004, 0.033, 2.41 $10^{-5}$ for the 970-MHz CW group, 9.70-GHz CW group, and 970-MHz PW group, respectively.

After an eight-month exposure, one observes a higher level in all exposed groups with respect to the sham-exposed group. The increase is largest in the 9.70-GHz CW group. Mean difference of 1.148, which is a 3.6% increase, between the sham-exposed group (32.23$\pm$0.6654) and 9.70-GHz CW group (33.37$\pm$ 0.6558) is statistically significant ($p = 2.35\ 10^{-8}$).

MCHC in the 970-MHz CW group (32.84 $\pm$ 0.5224) is 1.9% higher than in the sham-exposed group and differs: $p = 2.53\ 10^{-4}$. The 970-MHz PW group (32.55 $\pm$ 0.5450) shows a slight increase of 1.0%, which is not statistically significant: $p = 0.104$. After 11 months of exposure, the increase remains in the order of 3% ($p < 0.001$) for all exposed groups compared to the sham-exposed group.

After an 11-month exposure, one observes a higher level in all exposed groups in comparison with the sham-exposed group, which is of the order of 3%. Mean difference of 0.867 between the sham-exposed group (32.41 $\pm$ 0.7236) and 970-MHz CW group (33.28 $\pm$ 0.5690) is highly statistically significant ($p = 4.12\ 10^{-7}$). It corresponds to a 2.7% increase in MCHC. MCHC in the 9.70-GHz CW group (33.16 $\pm$ 0.4724) is 2.3% higher and differs in this significantly ($p = 1.26\ 10^{-5}$) from the sham-exposed group (32.41$\pm$0.7236). Comparing the 970-MHz PW group (33.05 $\pm$ 0.6328) with the sham-exposed group also shows a statistically significant difference ($p = 2.30\ 10^{-4}$). The 970-MHz PW group shows an increase of 2.0%.

### *D. Hemoglobin Concentration*

Hemoglobin concentration is expressed in g/100 mL. Fig. 6 shows that hemoglobin concentration has raised after a three-month exposure with almost equal amplitude in the three exposed groups with respect to the sham-exposed group. Differences between exposed groups are small, but the highest increase can be noticed in the 970-MHz CW group. Differences between mean values of the 970-MHz CW group (15.52$\pm$0.47) and sham-exposed group (15.13 $\pm$ 0.47) equal 0.39, which is 2.6% higher than in the sham-exposed group. Mean difference between the 9.70-GHz CW group (15.51 $\pm$ 0.56) and sham-exposed group is 0.38 (15.13 $\pm$ 0.47). The 9.70-GHz CW group shows a 2.6% higher hemoglobin concentration. The 970-MHz PW group shows a 2.5% higher hemoglobin concentration than the sham-exposed group.

Mean differences between the sham-exposed group and the three exposed groups are statistically significant with a $p$-value of 0.010, 0.011, and 0.014 for the 970-MHz CW group, 9.70-GHz CW group, and 970-MHz PW group, respectively.

### *E. Erythrocytes (RBC)*

Results of RBC count after a three-month exposure are represented in Fig. 7. Figures have to be multiplied by $10^6$ and stand for the number of erythrocytes per $\mu$L blood. All exposed groups have an RBC higher than sham-exposed group. The 970-MHz CW group (9.02 $\pm$ 0.39) differs to a larger extent from the sham-exposed group (8.75 $\pm$ 0.41), followed by the 9.70-GHz CW group (9.00 $\pm$ 0.43). Mean difference of 0.39 between the 970-MHz CW group and sham-exposed group is a 3.0% increase and is statistically significant ($p = 0.025$). Median of the 9.70-GHz CW group shows a higher (2.9%) RBC count than the sham-exposed group and is statistically significant ($p = 0.037$). RBC count in the 970-MHz PW group (8.873$\pm$0.3448) is 1.41% higher than in the sham-exposed group, which is not statistically different ($p = 0.475$).

### *F. Neutrophils*

After 18 months of exposure, statistically significant differences related to the neutrophils between exposed and sham-exposed groups are observed (Fig. 8). The mean value of the 9.70-GHz CW group (37.46 $\pm$ 10.86) is 34.7% higher than the

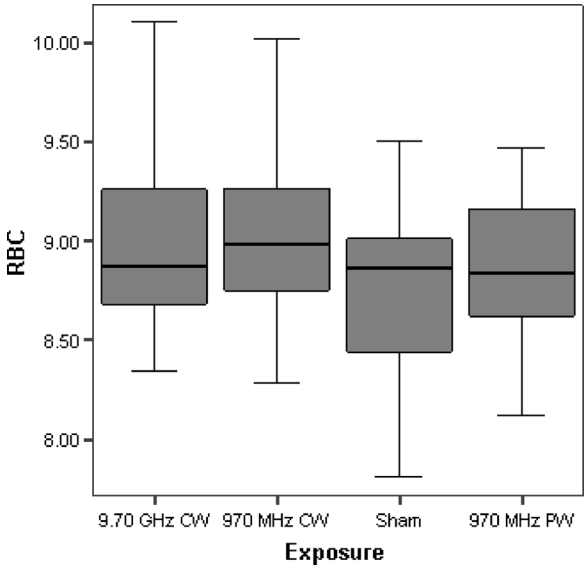


Fig. 7. Red blood cell count after three-month exposure.

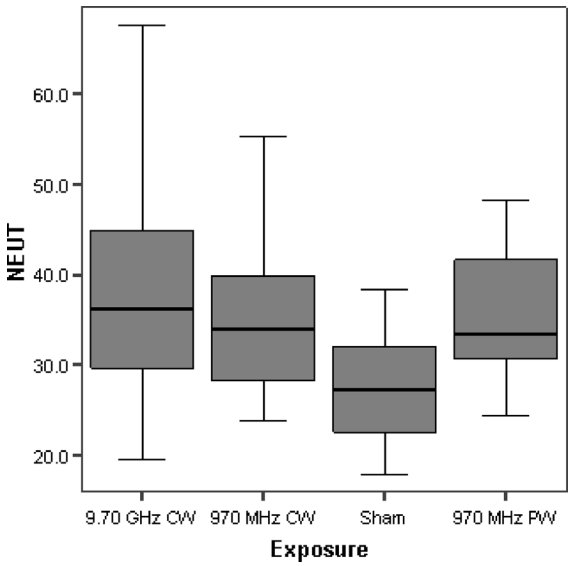


Fig. 8. Neutrophil count after 18 months of exposure.

mean value of the sham-exposed group ($p = 0.002$). In the 970-MHz CW group ($5.58 \pm 8.528$), the neutrophils showed an increase of 27.9% ($p = 0.016$).

The mean value of the 970-MHz PW group ($27.81 \pm 6.506$) is 26.4% higher than in the sham-exposed group ($p = 0.001$).

### G. Lymphocytes

After 18 months of exposure, statistically significant effects between exposed and sham-exposed groups are reported (Fig. 9). The mean value in the 9.70-GHz CW group ($56.10 \pm 11.34$) is 15.1% lower than in the sham-exposed group ($66.09 \pm 6.709$). This difference is statistically significant ($p = 2.47\ 10^{-4}$). The lymphocyte count in the 970-MHz CW group ($57.50 \pm 9.130$) is 13.0% lower than in the sham-exposed group ($p = 0.006$), whereas in the 970-MHz PW group ($58.41 \pm 7.229$), it is 11.6% lower ($p = 0.008$).

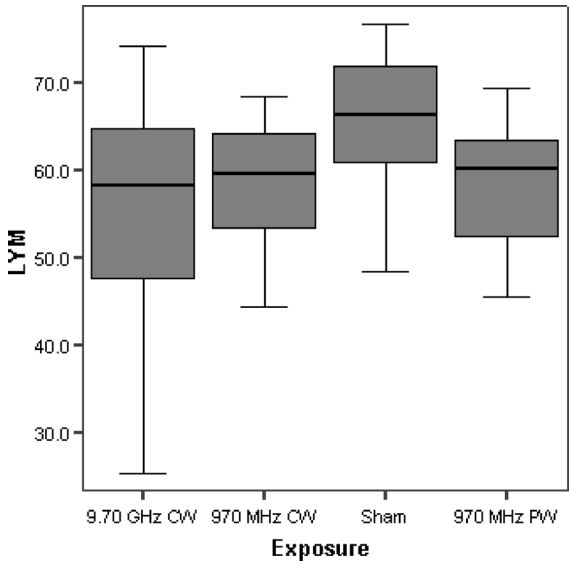


Fig. 9. Lymphocyte count after 18 months of exposure.

TABLE I
MORTALITY RATE IN THE FOUR EXPOSED GROUPS AFTER 21-MONTH EXPOSURE

| | Survival | Dead | Mortality% | Survival% |
|---|---|---|---|---|
| Sham-exposed | 24 | 7 | 22.5% | 77.5% |
| 970 MHz CW | 19 | 12 | 38.7% | 61.3% |
| 970 MHz PW | 19 | 12 | 38.7% | 61.3% |
| 9.70 GHz CW | 20 | 11 | 35.5% | 64.5% |
| Σ Exposed | 58 | 35 | 37.6% | 62.4% |

### H. Mortality

Physiological data differ not only between rodent species, but even between different strains of rats used in animal studies. For this reason, we took as one reference for mortality comparison the longevity studies run by the Charles River Laboratories where we purchased our rats. We complementary compared mortality rates with those mentioned in the Charles River Laboratories document [48].

At this time, we cannot provide a comprehensive report of all of the biological endpoints measured, together with a review of the pathology reports on the deceased animals. Pathology investigations are going on. They are far from being completed and they will certainly take more than a year. Indeed, due to the increased rate of mortality in exposed rats, a new question has been raised: is this due to premature aging? This question complicates the process of pathological investigations.

In animal studies, the effect of an agent on mortality is one of the first parameters to investigate. We first compared the mortality rate in all exposed groups to that of the sham-exposed group at the end of the exposure period, i.e., after 21 months of exposure, at the age of 25 months. Rats that had been subject to early euthanasia for ethical reasons have been taken into consideration. The mortality rate is displayed in Table I. It is higher in exposed groups than in the sham-exposed group: 37.6% versus 22.5%, respectively. When differentiating between the three exposed groups, the highest mortality rate is observed at 970-MHz CW (38.7%) and 970-MHz PW (38.7%), which is 72% higher

TABLE II
MORTALITY RATE IN EXPOSED VERSUS SHAM-EXPOSED GROUPS AT THE AGE OF 24 MONTHS

| | Survival | Dead | Mortality% | Survival% |
|---|---|---|---|---|
| Sham-exposed | 25 | 6 | 19.3% | 80.7% |
| 970 MHz CW | 21 | 10 | 32.3% | 67.7% |
| 970 MHz PW | 21 | 10 | 32.3% | 67.7% |
| 9.70 GHz CW | 23 | 8 | 25.8% | 74.2% |
| Σ Exposed | 65 | 28 | 30.1% | 69.9% |

TABLE III
MORTALITY RATE IN THE FOUR EXPOSED GROUPS AFTER A THREE-MONTH NONEXPOSED PERIOD FOLLOWING 21-MONTH EXPOSURE

| | Survival | Dead | Mortality% | Survival% |
|---|---|---|---|---|
| Sham-exposed | 22 | 9 | 29.0% | 71.0% |
| 970 MHz CW | 16 | 15 | 48.4% | 51.6% |
| 970 MHz PW | 13 | 18 | 58.1% | 41.9% |
| 9.70 GHz CW | 12 | 19 | 61.3% | 38.7% |
| Σ Exposed | 41 | 52 | 55.9% | 44.1% |

than in sham-exposed group. Mortality rate at 9.70-GHz CW is 35.5%, which is 58% higher than in the sham-exposed group.

The 22.5% mortality rate for sham-exposed rats at the age of 25 months is standard. Charles River Laboratories performed many tests on survival rates on the type of rat we used. Based on ten longevity studies run during 24 months, typical survival rate data have been obtained for male rats [48]. Mean survival rate for male Han rats after 24 months is 78.8%.

In order to compare rat survival rate in our experiment to the survival rates in the Charles River Laboratories reference group, we calculated the mortality rate at the age of 24 months in our group of rats. This can be found in Table II.

An 80.7% survival rate in the sham-exposed group is normal according to the Charles River Laboratories reference data. A survival rate of 69.9% in the exposed groups is not common, as can be noticed in [48]. The exposed groups show survival rates situated beneath the mean survival rate (78.8%) of nontreated Han rats. These findings make it plausible to us to suggest that the exposure could have had an effect on the overall survival of our rats.

After 21 months exposure, surviving rats have been kept alive, but without daily exposure to microwaves. After a three-month post-exposure period, i.e., at the age of 28 months, we compared mortality rates in exposed and sham-exposed groups, respectively (Table III).

It is most interesting to observe that there is a much larger difference between exposed and sham-exposed groups mortality at the age of 28 months than at the age of 25 months: 55.9% versus 29.0% at 28 months, respectively, instead of 37.6% versus 22.5% at 25 months, respectively. This average mortality rate in the exposed groups at the age of 28 months represent an increase of 93% with respect to the sham-exposed rats, to be compared to an increase of 67% at the age of 25 months. Mortality rate increases, compared to sham-exposed group, for 970-MHz CW, 970-MHz PW, and 9.70-GHz CW groups are 48.4%, 58.1%, and 61.3%, respectively. To consider the mortality in the course of the entire exposure period, a Kaplan–Meier survival analysis [45] is performed to compare mortality in exposed groups to mortality in the sham-exposed group. It is an estimate of the survival function from lifetime data and is used to measure the fraction of rats living for a certain amount of time after the experiment has started. A statistically significant effect ($p = 0.017$) is observed (log-rank test [45]) between sham-exposed and 9.70-MHz CW exposed rats. The difference in survival percentage between sham-exposed and 970-MHz PW exposed rats can be characterized as nearly statistically significant ($p = 0.082$). We did not find a statistically significant difference between the mortality in the sham-exposed and 970-MHz CW exposed group ($p = 0.349$).

## IX. DISCUSSION

It should be noted that some of the statistically significant effects are small. We found an increase of about 3% in the RBC count of all exposed groups compared to the sham-exposed group. A similar result was found regarding the hemoglobin concentration where the exposed groups exhibit a nearly 3% increase versus the sham-exposed group. Also for the MCHC, one observes an increase (2%) in all exposed groups with respect to the sham-exposed group. As the MCHC measures the average concentration of hemoglobin in an RBC, it is plausible that an increase in erythrocytes leads to an increase in MCHC. This is consistent with our findings. Although the reported effects are small and of no biological significance, it is worthwhile to mention that some groups found a similar trend. Busljeta *et al.* [49] observed an increased erythrocyte count in peripheral blood of male Wistar rats after exposure to 2.45 GHz (2 h, daily, 1–2 W/kg, 15 days). Heikkinen *et al.* [50] exposed mice for 78 weeks (1.5 h/day, five days/week) at 1.5 W/kg (902.5 MHz) and concluded that RF exposure did not affect hematological parameters, except for hemoglobin concentration that the authors regarded as a chance effect because no other hematological parameters of RBCs were changed.

The main objective of this study consists in evaluating if long-term exposure to low-level microwaves is able to produce biological effects on the long run. We observed a highly statistically significant increase in mortality in the 9.70-GHz CW exposed group. Several hypotheses can be formulated. We started the experiment with young rats. Tissues of four-month-old rats are still in a developing stadium. It might be hypothesized that young rats are more vulnerable to microwaves as possible alterations on the molecular level are not fully compensated. A biological effect that is not compensated may be concretized in damage in biological structures leading to a health effect, which on its turn can influence mortality. In the literature, there are very few long-term (21 months of daily exposure and more) studies reporting survival data in rats, as mentioned in Section I. It is even harder to find mortality studies where rats have been exposed to frequencies beyond 2.45 GHz. Adey *et al.* [23] reported no effect on the survival of rats exposed to 836-MHz frequency modulation (2 h/day, four days a week, 24 months). In contrast with our study, the total exposure period was about half of which we considered. Besides, the continuity of the exposure was interrupted each time after four days, which allowed the exposed rats to compensate for an eventually occurred biological effect. Toler *et al.* [25] and Frei *et al.* [26] exposed mice for 21 months (22 h/day) and 18 months (20 h/day), respectively. No decrease in survival was reported. Toler *et al.*, however, submitted mice to a substantial lower frequency (435 MHz) than we did. In a

long-term study of rats exposed to pulsed 2.45-GHz fields, Chou *et al.* [27] reported no statistical significant effect on survival rates; although survival was not affected, they found—if all primary malignancies were analyzed together—a statistically significant increase in tumors in the microwave exposed group relative to controls. The incidence of benign tumors did not differ.

## X. Conclusions

After three- and eight-month exposures, we found a statistically significant difference of about 20% between the 970-MHz CW group and sham-exposed group regarding monocytes. Exposure may probably have worked as a trigger and influenced the immune system, which reacted to this stressor by increasing the percentage of monocytes in the peripheral blood circulation. Further research related to the biological relevance of this finding is needed.

Blood hemoglobin concentration is another parameter for which we found a statistically significant effect in the 9.70-GHz group. We found no explanation for this increase.

The most obvious effect we detected is the increase in mortality rate of exposed groups with respect to the sham-exposed group after 21-month exposure at the age of 25 months. This increase even grows when observing rats until the age of 28 months: mortality in exposed groups then reaches almost twice the value observed in the sham-exposed group.

Animals, just as humans, die when body defenses cannot compete anymore with the environment aggressors in the wide sense of the word, i.e., bacteria, viruses, cancer cells, blood pressure, temperature, etc. The immune system plays a crucial role in upholding the general health condition: it is a primary determinant of survival. In our opinion, the question whether long-term microwave exposure of rats may effect longevity is far from answered today. In our study, two out of three microwave exposed groups show a statistically significant (9.70-GHz CW) or nearly statistically significant (970-MHz PW) decrease in survival. This trend is interesting.

## Acknowledgment

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