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An Epidemiological Study on Low-level 21-month Microwave Exposure of Rats

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Glossary

Symbols used in the manuscript

Ca^{2+}	- Calcium ions
D	- Largest characteristic dimension of the radiating antenna
Da	- Dalton
e	- Euler's number (2,718…)
$\overline{E}$	- Electric field vector
f	- Frequency
$\overline{H}$	- Magnetic field vector
K^+	- Potassium ions
m	- Mass
P	- Electromagnetic power
$\overline{P}$	- Poynting vector
R	- Boundary
δ	- Skin depth
λ	- Wavelength
μ	- Tissue magnetic permeability
ρ	- Density
σ	- Tissue conductivity
V	- Volume element

Acronyms and Abbreviations Used in the Manuscript

2G	- Second generation
3G	- Third generation
ACTH	- Adrenocorticotropic hormone
ANOVA	- Analysis of variance
BBB	- Blood-Brain Barrier
CORTICO	- Corticosterone
CW	- Continuous Wave
DAB	- Digital Audio Broadcasting
DAB-T	- Digital Audio Broadcasting Terrestrial
DCS	- Digital Communication System
DECT	- Digital Enhanced Cordless Telephone
DNA	- Deoxyribonucleic acid
DVB-T	- Digital Video Broadcasting Terrestrial
EDGE	- Enhanced Data rates for GSM Evolution
EEG	- Electroencephalogram
EHS	- Electromagnetic Hypersensitivity
ELF	- Extremely Low Frequency
ELISA	- Enzyme-Linked ImmunoSorbent Assays
EOS	- Eosinophils
E-plane	- Electric Field
FM	- Frequency Modulation
GPRS	- Global Packet Radio Service
GSM	- Global System for Mobile communications
HCT	- Haematocrit
HE	- Haematoxyline-Eosine
HF	- High Frequency

HGB	- Haemoglobin
H-plane	- Magnetic Field
ICNIRP	- International Commission on Non-Ionizing Radiation Protection
IEEE	- Institute of Electrical and Electronic Engineers
kbps	- Kilobits per second
LAN	- Local Area Network
LYM	- Lymphocytes
M	- Mean
MCH	- Mean Corpuscular Haemoglobin
MCHC	- Mean Corpuscular Haemoglobin Concentration
MCV	- Mean Corpuscular cell Volume
MOC	- Monocytes
NEUT	- Neutrophils
ODC	- Ornithine Decarboxylase
PCR	- Polymerase Chain Reaction
PHA	- Phytohaemagglutinin
pps	- Pulses per second
PW	- Pulsed Wave
RBC	- Red Blood Cell
RETIC	- Reticulocytes
RF	- Radiofrequency
RLAN	- Radio LAN
rms	- Root-mean-square
SA	- Specific Absorption
SAR	- Specific Absorption Rate
SD	- Standard Deviation
SPSS	- Statistical Packages for Social Sciences
TDMA	- Time Division Multiple Access
TETRA	- Terrestrial Trunked Radio

UCL	- Université catholique de Louvain
UMTS	- Universal Mobile Telecommunications System
VHF	- Very High Frequency
WBC	- White Blood Cell
WHO	- World Health Organization
WiFi	- Wireless Fidelity
WiMAX	- Worldwide Interoperability for Microwave Access
WLAN	- Wireless LAN

Introduction

The last decade new technologies in personal communication have led to an increased exposure of the public to electromagnetic waves. Microwaves are used extensively both in the private atmosphere as on the work place. We are all subject to electromagnetic fields produced by cellular telephony, wireless communication devices, radars, *etc*. In the perspective of human health, millions of people are nowadays exposed to low-level microwaves, with the prospect that these newly evolved patterns will continue on a lifelong basis.

These recent electronic devices and systems overwhelm the natural electromagnetic environment with more intense fields. This growth of radiofrequency (RF) fields is further complicated by the advent of digital communication techniques. In many applications, these microwave fields are systematically interrupted (pulsed) at low frequencies.

Microwave applications in the industry have increased over the past 40 years. Microwaves have become commonplace on the occupational scene. Throughout the manufacturing industries, new processes are all the time being developed using latest microwave technology. Workers may be continuously exposed to fields from a plethora of sources. In particular, this is certainly true for military personnel in operation. Besides, it is not uncommon to observe a high concentration of communication devices – mobile or ground-based – (satellite communication, high frequency (HF) transmitting antennas, radars, …) implanted on a small compound in the immediate surrounding of personnel.

From the point of view of biological evolution, the omnipresent electromagnetic radiation due to the proliferation of man-made sources constitutes a very recent physical factor in the environment. The last ten

years, everyone is exposed to a complex mix of electromagnetic fields at higher intensities than ever before.

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 the biological effects of long-term exposure to electromagnetic waves remains unresolved. With the expanding use of microwaves, the general population is becoming increasingly aware of and even concerned about the potential adverse effects of these electromagnetic fields of different frequencies and transmission modes.

Therefore, the WHO stimulates further investigation about the biological long-term effects of low-level exposure to microwaves.

That is what this thesis is about: the work concentrates on the biological effects observed in an animal study with rats that have been exposed to low-level microwaves during almost all their lifetime. The basic input comes from periodical blood samplings which have been performed during the entire exposure period together with the mortality study at the end of the experimental period.

This thesis is divided in four parts:

In the first part the complete format and the fundamentals of the experiment will be described in detail.

The frame of the study will be designed in chapter one.

In chapter two, today's radiofrequency environment will be presented and the choice for the frequencies used in the experiment will be explained. A section will be devoted to possible biological implications of continuous wave *versus* pulsed wave exposure. Thermal and low-thermal considerations

about exposure parameters will be made in the next section. Experiments showing or not an influence of microwave exposure on the integrity of the blood-brain barrier will be shortly discussed in a following section.

An exhaustive description of our experiment will be the main subject in chapter three. Practical solutions for encountered problems will be presented together with the selection of the haematological parameters which will be investigated after the periodical blood samplings.

In the second part, the statistical analysis of the laboratory results regarding the haematological parameters will be performed. Period by period, differences observed between exposed and sham-exposed groups will be tested on their statistical significance.

In the third part, preliminary results of our behavioural study on rats regarding possible cognitive effects of microwaves will be presented. It is an onset for further research in this domain. The second chapter will deal with a mortality study applied to the four populations of rats that have been almost life-long exposed to low-level microwaves. Not so many studies have included a mortality study in their design because of the long-term dedication to this labour-intensive scientific project.

Part four is in the line of the mortality study; an anatomopathological study of a sample of rats taken out of each exposed group will be performed. The histopathological examination of all rats goes beyond the scope of this thesis.

The summary of the findings will be written in the final conclusion, which is also an impulse for further discussion and scientific study.

Part I Experiment

Chapter 1 Description

1.1. Frame of the Study

In this study we examine the influence of exposure to low-level microwaves. Microwaves are part of the radiofrequency (RF) spectrum which is definied as extending from 3 kHz to 300 GHz. Microwave frequencies lie between very high frequency (VHF) radiowaves and the far infrared and occupy the part of the electromagnetic spectrum between 300 MHz and 300 GHz.

Last years, people are more frequently exposed to radiofrequency radiation and microwaves emanating from cellular phones, base stations, wireless applications in rather new technologies as Wireless Fidelity (WiFi), Bluetooth, …. The thermal effects of those frequencies are well understood. Much less is known about the possible low-thermal effects on the long run.

The omnipresense of radars at the operational theatres of the Belgian Defence and the expanded use of modern technologies based on microwaves in the military environment constitute another impulse for conducting this scientific study. Some cancer incidences among military personnel are said to be attributed to radar exposure.

The World Health Organization (WHO) encourages well-conducted studies, targeted at specific endpoints using established animal models, which will help to formulate a more complete assessment of the biological effects and the potential health impact of low-level microwaves [1, 2].

These studies will assist in providing improved standards and guidelines for human exposure.

1.2. Advantage of an Animal Study Compared to a Human Epidemiological Study

Human and pure epidemiological studies are most difficult to interpret. All epidemiological research suffers from the fact that it is an observational, rather than an experimental, branch of science. Moreover, exposure assessment in the case of epidemiological research has proven to be very complex and has usually been inadequate to answer refined questions, sometimes to the point that the existence of meaningful exposures is in question. Besides, it is rare that one or even a few human epidemiological studies can be conclusive. Laboratory studies of human beings also need a set of similar studies in order to make useful interferences that are relatively free of errors in a particular protocol or subtle cues to subjects who are believed blind to experimental conditions. Questionnaires are particularly not easy to execute without the possibility of unintended influences on the subjects, making interpretation of data at least as difficult as for other human studies. In addition it is most difficult to assess the level of exposure of subjects in a human epidemiological study. Besides, the exposure to environmental factors other than the independent variable, *e.g.* toxic products of which one is unaware, can influence the final outcome of the study. These limitations should not deter the development of a human studies database but do imply that one needs to develop a complementary database of animal studies.

For these and other reasons, we have chosen for an experimental study with laboratory animals. In our experimental study, the exposure level to which the animals are subject is univocally determined before the start of the experiments. This constitutes a great advantage compared to a human epidemiological study where the initial situation is much less defined.

1.3. Description of the Experiment

The present work is a long-term (21 months of exposure) experimental epidemiological study on rats, aiming to trace possible physiological and cellular changes in 124 *Wistar* albino rats due to low-thermal microwave exposure at a frequency of 970 MHz continuous wave (CW), 970 MHz pulsed wave (PW) and 9.70-GHz CW. There are in total four groups of 31 rats, the sham-exposed group included.

This work is mainly focused on the biological effects of microwaves on monocytes, lymphocytes, eosinophils, erythrocytes, and haemoglobin. Other biological endpoints are among others stress induced hormones as adrenocorticotropic hormone (ACTH) and corticosterone. At the end of the experiment, the surviving rats are sacrificed and an anatomopathological analysis on liver, lungs, kidneys, heart, thymus, bladder, spleen, brain tissue, testes, and gastro-intestinal track will be performed. In the framework of this thesis, only a few rats are examined. A mortality study comparing the surviving rates between the exposed groups is performed.

The number of animals to be used in the experiment is based on a certain statistical basis, stating that a number of minimal 20 observations for each group of animals gives rise to a normal distribution [3]. Working with normal distributions facilitates the statistical analysis afterwards. Against this statistical background, we considered taking 31 rats for each of the exposed groups; this also takes into account possible losses. There is also a practical and financial reason that has limited our population to this number of rats.

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Chapter 2 The Radiation Environment and Observed Effects

In this chapter we firstly describe emerging microwave devices and techniques together with the utilized frequencies and we explain on which base we have selected the microwave frequencies being used in our experiments. Further on we report on different effects of electromagnetic fields that have been observed as a function of frequency, exposure mode, and power. Finally we present an extensive section on the blood-brain barrier with its anatomical and physiological particularities before giving an overview of recent findings in literature regarding possible alterations in its permeability under the influence of microwaves.

2.1. Today's Radio Frequency Environment Used as a Basic Criterion for the Selection of Frequencies in Our Study

2.1.1. The Radio Frequency Environment

Hardly any phenomenon in the modern environment is as ubiquitous as electromagnetic fields. Electronic devices communicating *via* a wireless network and new microwave-based technologies have become more and more part of our daily life. In our countries, cellular telephony has emerged as the wireless technology of choice for basic telephone service.

Worldwide, the second generation (2G) digital systems are dominated by the Global System for Mobile communications (GSM) and the Digital Communication System (DCS)-1800. GSM is the digital mobile communication standard agreed by the European countries, network operators and equipment manufacturers in the 1980s. The first commercial

GSM was launched in Belgium on January 1st, 1994. The number of users growed fundamentally faster than it was expected at the introduction of the system. Therefore, a second frequency band centered on 1800 MHz had to be provided and is occupied by DCS-1800.

GSM is a time-division multiple-access (TDMA) system, operating at a carrier frequency situated around 900 MHz (880 MHz – 959 MHz) with a characteristic burst frequency of 217 Hz. For many years ahead, millions of people will continue to use basic GSM. Typical output powers are 30 W and 2 W for the base station and the handset, respectively.

The second generation telecommunication systems further include the indoor wireless telephone also referred to as Digital Enhanced Cordless Telephone (DECT), and TErrestrial Trunked RAdio (TETRA).

In 1988, with the introduction of DECT, one decided on a European solution for cordless telecommunication, which employs ten carrier frequencies between 1.88 GHz and 1.90 GHz. DECT was originally developed to replace the early cordless telephone standards. However, this system goes far beyond that of its analogue predecessors. The market opportunity is in residential and business site mobility and in that sense it provides a non negligible contribution to the electromagnetic fields to which people are daily exposed. Typical maximum output power of the DECT base station is 250 mW.

TETRA is a rather new digital-radio technology, mainly meant for emerging services, police, military, other security services, and the like. It differs from GSM by also having a direct mode, from terminal-to-terminal. The idea of TETRA is the trunking facility (multiple base transceivers networked to offer wide area coverage), which provides a pooling of all radio channels, which are allocated on demand to individual users, in both voice and data modes. A power of maximum 40 W (typical 15 W) for the base station and

maximum 30 W (typical 1 W) for mobiles is defined. It is a TDMA system, at frequencies from 380-383 MHz and 390-393 MHz in Europe, allocated for emergency services. Several smaller frequency bands between 385 MHz and 921 MHz are reserved for civilian applications. The burst-mode frequency in the TETRA system is 17.65 Hz, which is much lower than for the other systems, and has given rise to some speculation on possible adverse biological effects.

An overall picture with sources of radiofrequency emitting devices used in modern communication is presented in Figure 2.1, together with the appropriate carrier frequencies.

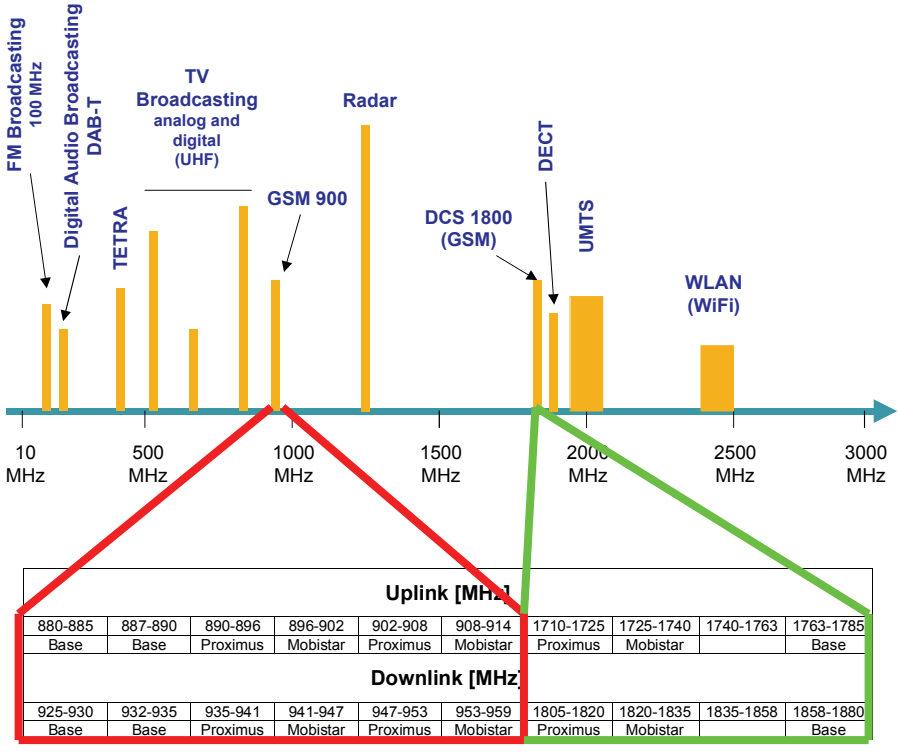

Figure 2.1: Radiofrequency electromagnetic spectrum with typical communication applications.

One of the apparent market drivers is the need for wireless data, and basic GSM, originally designed for speech, is limited to 9.6 kilobits per second (kbps). In order to carry more data, new systems are gradually being introduced in the GSM family. Presently, we are in the midst of the transition from the 2.5 generation (2.5G) towards the third generation of cellular systems, with the promise of larger data rates, with multimedia services. One is Global Packet Radio Service (GPRS) – so-called packet-switched data – with rates as high as 170 kbps, and another is the Enhanced Date rates for GSM Evolution or better known under the acronym EDGE, with up to 384 kbps using higher-order modulations, both achieving high data rates by combining several time slots. This means that the effective transmission duty cycle could be 4/8 of the time, instead of 1/8, as is the case for standard GSM. The maximum nominal power is still 2 W, so theoretically, the mean power could approach 1 W. It is assumed, however, that in most cases the output power will be much less.

The third generation (3G) of mobile communication systems is characterized by a higher data transfer rate (> 2 Mbps) than the second-generation systems and offer packet-switched as well as circuit-switched voice services with a host of new features that are user-friendly and improved performance for transfer of data, voice, and video. Examples of 3G cellular-system technologies include Universal Mobile Telecommunications System (UMTS). The frequency bands of 1885-2050 MHz and 2110-2200 MHz have been allocated for UMTS. The cited technologies GSM, UMTS and TETRA offer a manifold way for communications.

Local Area Networks (LAN) allow data communication between computers within a confined region, *e.g.* a campus or a large building. Until today, most of these networks have been using wire connections, but nowadays new wireless applications using radiofrequencies have been introduced and will be more and more widely asked. These LANs are called Radio LANs or

RLANs, sometimes also called Wireless LANs (WLAN). Each node of the network is equipped with a small radio transceiver and interconnecting cables are no longer necessary, simplifying the use of the network and allowing mobility of its components (portable computers, …). WiFi is standardized for limited-range communications between devices, like laptop computers. Carrier frequencies for such LANs depend on the geographical region, but are typically higher than for cellular systems (2.45 GHz to 3 GHz, 5 GHz, and 17 GHz). These are rather unexplored frequencies, from a dosimetric and biological viewpoint. The Worldwide Interoperability for Microwave Access (WiMAX) technique is a long-range version of WiFi, which makes it possible to achieve a broad band connection to the internet from any location by means of microwaves. In Belgium, frequencies from 3.5 to 23 GHz are reserved for this microwave application. At this moment already 20 base stations are installed for pre-WiMAX, typically transmitting a power of 63 W.

Some short-range radio systems, like Wireless Fidelity (WiFi) and Bluetooth, are emerging, with the promise of high data rates over short distances, competing with the cellular systems for stationary applications.

Bluetooth is a hot topic among wireless developers. It is considered to be the low cost key technology for the short range communication of neighbouring equipment. It is designed to allow low bandwidth wireless connections. Bluetooth is of special interest, since it can be expected that it will be embedded in most digital devices of the future, from mobile phones to cameras, printers, household devices, and so on. This is a general trend: rather low exposure from the individual device, but many of them. The nominal power level for basic use is 1 mW, which provides a range of about 10 m. Bluetooth works in the unlicensed band at 2400 – 2438.5 MHz. The transiever utilizes frequency hopping to reduce interference and fading.

A non exclusive list of new wireless communication systems which have appeared in daily life during the last 5 years is shown in Table 2.1.

Name	Carrier frequency (round figures)
TETRA	400 MHz
GSM	900 MHz
DCS	1800 MHz
DECT	1900 MHz
UMTS	2000 MHz
WLAN (WiFi)	2400 MHz
Bluetooth	2400 MHz
WiMAX	3500 MHz – 23 GHz (Europe); 2400 and 5000 MHz (USA)

Table 2.1: Common civilian contributions to today's RF environment.

The established overview of today's communication systems and their characteristics played an important role in the selection of the target frequencies in our experimental design. This will further be developed in section 2.1.2.

2.1.2. Selection of Frequencies for the Experiments

We have selected two frequencies around which our experiments are built up: 970 MHz and 9.70 GHz. The reason for this choice is twofold: the technical reality of today's most utilized frequencies in public and private telecommunication (as explained in section 2.1.1) and a physical-biological interaction mechanism as will be explained further on in this section.

Computations of absorbed energy as a function of frequency and body size have been made for using homogeneous muscle material to serve as an index of Specific Absorption Rate (SAR) in animals and to serve as a guide for

extrapolating data from experimental animals to human beings, particularly with regard to averages of absorbed energy. SAR is the ratio of absorbed power to absorbing mass and is defined [1] as the incremental electromagnetic power (dP) absorbed by an incremental mass (dm) contained in a volume element (dV) of given density (ρ):

$$\text{SAR} = \frac{dP}{dm} = \frac{dP}{\rho dV} \tag{2.1}$$

Instantaneous power density P is the product of the vectors of the electric field E and the magnetic field H. Because sinusoidally varying signals in time are often used, a time-averaged power density that is one half of the peak values is encountered. For electrically conducting media such as biological tissues, the rate of energy dissipated per unit volume at a certain point is given by:

$$\frac{E_i^2}{2}\sigma \ (\text{W/m}^3) \tag{2.2}$$

where E_i is the peak value of the internal electric field strength in volts per meter and where σ is the electrical conductivity of the medium in Siemens per meter. If specific absorption rate is required, the quantity (2.2) needs to be devided by the mass density of the tissue. Hence, the SAR is expressed as:

$$\text{SAR} = \frac{E_i^2}{2\rho}\sigma = \frac{E_{rms}^2}{\rho}\sigma \tag{2.3}$$

where ρ is the mass density of the tissue in kilograms per cubic meter and E_{rms} is the effective value or root-mean-square of the internal electrical field strength, *i.e.* the square root of the mean value of the square of the internal electrical field strength [2, 3].

While the average SAR is defined as a ratio of the total power absorbed in the exposed body to its mass, the local SAR refers to the value within a defined unit volume or unit mass, which can be arbitrarily small [2]. Partial-body SAR averages SAR over any 1 gram (approximately a cube measuring 1 cm on each side) or 10 grams tissue of the body, depending on the RF safety standard in question. These 1 g or 10 g of tissue are intended to be a mass of contiguous tissue with nearly homogeneous electrical properties. The smaller the tissue mass is (1 g in place of 10 g) in which the RF is absorbed the greater the temperature rise is. Therefore, a local SAR limit averaged over 1 g of tissue is much harder to meet than a SAR limit averaged over 10 g of tissue.

Such local SAR values are necessary to evaluate and limit excessive energy deposition in small parts of the body resulting from special exposure conditions. Even when the whole body of a biological subject is exposed to plane-wave (far field) RF exposure, local internal hot spots are induced. Therefore, most contemporary experimental dosimetry is performed by taking many SAR data points within a biological subject or model. These data can be integrated later to obtain a coarse approximation of whole-body-averaged SAR. In specifying a contiguous mass of tissue, it is recognized that this concept can be used in computational dosimetry, but may present difficulties for direct physical measurements. In practice, direct measurements of SAR are feasible only under laboratory conditions.

It is noteworthy that for a given incident field the SAR for humans may be either higher or lower than for animals, depending on the frequency. This is graphically represented in Figure 2.2.

For example, at 70 MHz, the average SAR is the highest for humans, having a value of 0.25 W/kg for an incident power density of 10 W/m² while the average SAR for a rat is 0.0125 W/kg. In contrast, at 700 MHz, the average

SAR is the highest for rats (0.8 W/kg) [4]. The corresponding value for humans at that frequency is about 0.03 W/kg; this is less than 1/25. It is thus extremely important to take into account the body size and operating frequency to establish any relationship between the biological effects that arise in the laboratory and corresponding effects that might occur in humans at a given incident power density.

The frequency for maximum absorption (resonance frequency) depends on the subject and its orientation with respect to the incident field. In general, the shorter the subject, the higher the resonance frequency and *vice versa*.

The physico-biological interaction mechanism is based on the fact that the resonant frequency of a rat is in the order of 700 MHz, whereas the resonant frequency of an average human is about 70 MHz [5]. This can be observed in Figure 2.2. Consequently, if the ratio 1:10 is to be maintained, a frequency in the range of 9 GHz should be applied in our experimental setting, starting from one of the frequencies of the GSM cellular telephone (900 MHz).

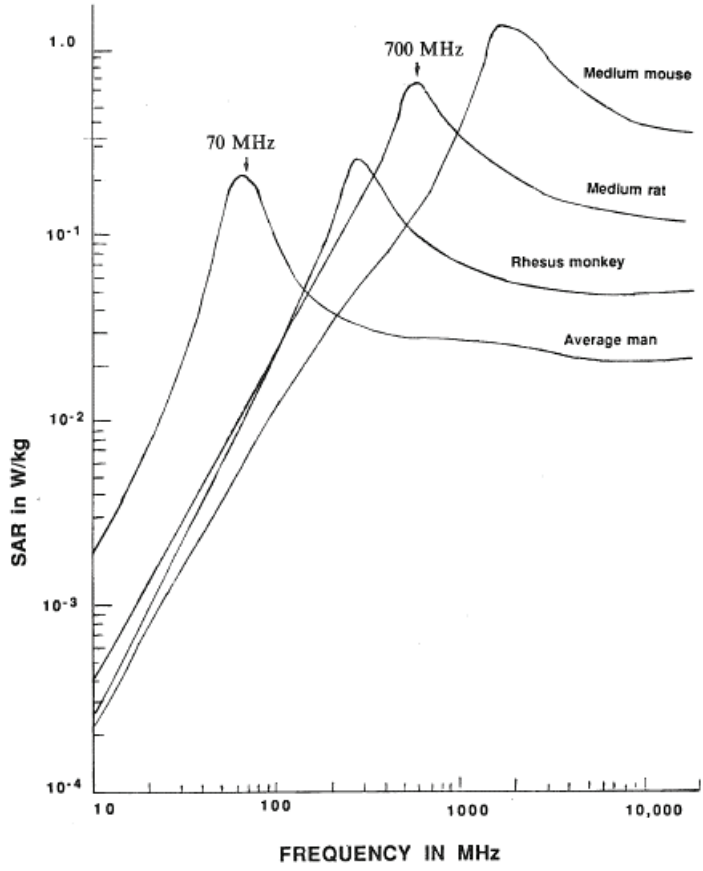

Figure 2.2: Whole-body average SAR of prolate spheroidal models of 4 species exposed to 10 W/m² with the electric field vector parallel to the long axis of the body. Adapted from Durney *et al.* [6].

By the selection of the experimental frequencies, one has to take into account not only the aspect of resonance, but also the concept of penetration depth has to be considered. Penetration is limited by what is termed the skin effect, characterized by the skin depth. The depth to which microwaves can penetrate biological tissues is primarily a function of the electric and magnetic properties of the tissue and the microwave frequency. As the

transmitted waves penetrate into tissue, energy is extracted from the wave and absorbed by the medium, resulting in a progressive reduction of the power density of the wave as it advances in the tissue. This reduction is quantified by the depth of penetration - which is the distance in which the power density decreases by a factor e^{-2} - and the related skin depth (e = 2.718 is the base of the natural logarithm). The skin depth is the distance over which the field decreases to 1/e (= 0.368) of its value at the interface [7].

As defined by the equation

$$\delta = \frac{1}{\sqrt{\pi f \mu \sigma}} \text{ m} \tag{2.4}$$

with δ the skin depth, f the frequency of the incoming field, μ the tissue magnetic permeability, and σ the tissue conductivity, one can observe that the skin depth decreases inversely proportional with the square root of the frequency [8]. At a depth of 3 δ, the field amplitude is only 5% of its amplitude at the interface and the corresponding power is only 0.25%. For all practical purposes, electromagnetic waves can be said to be completely attenuated at depths equal to 5 δ. At this depth the field amplitude reduces to 1% and the corresponding power to 10^{-4} [7].

At 900 MHz the depth at which the power reduces to 1% is 3 cm in human tissue. At a 10 times higher frequency, the skin depth decreases with a factor 1/3.16. As a consequence, the depth at which the power reduces to 1% is about 1 cm at 9 GHz.

Figure 2.3 represents the variation of the power absorbed inside a human body as a function of penetration depth at several frequencies.

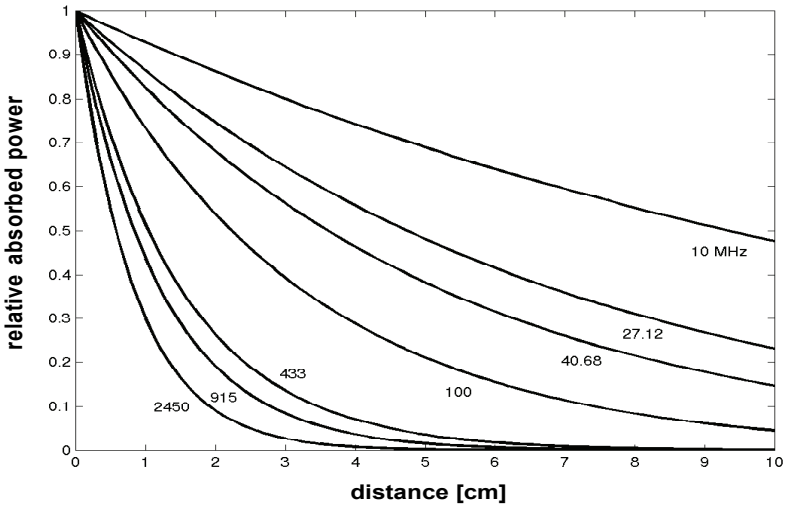

Figure 2.3: Power absorbed in muscle tissue as a function of the skin depth at various frequencies (frequencies expressed in MHz) [7].

The average penetration depth at the head of the rat at 9.4 GHz, if the dielectric characteristics of the various tissues are to be considered, according to the Brooks Air Base Model [9], is approximately 5 mm. In comparison, the penetration depth in the case of a GSM cellular telephone (carrier frequency, 900 MHz) is in the order of 55 mm and for the most commonly used WLAN, 19 mm.

As described in 2.1.1, a variety of wireless communication devices operate at frequencies between 0.8 GHz and 2 GHz. The 970-MHz frequency has been selected as one of the two frequencies to be used in our experiments as it is a frequency situated between both the ubiquitous 900 MHz and 1800 MHz from the GSM and the DCS-1800, respectively. Moreover, UMTS, with its carrier frequency of 2 GHz, together with DECT, are operating in the neighbourhood of 1 GHz too. Besides, there is the wide

spread presence of WLAN in public places like libraries, airports, schools and train stations, using a carrier frequency of 2.4 GHz.

In the military, several radars are working at carrier frequencies in the 1-GHz range. Radars are among others used in air-defence systems, for battle field surveying, air traffic control, long range surveillance, and aboard ships, where space is relatively limited and a high concentration of RF devices can be observed.

The reason for including the 9.70-GHz frequency in our experiments has among others to be linked to the fact that military radars have carrier frequencies around 1 GHz, 10 GHz and 16 GHz. Both continuous wave and pulsed wave are frequently used in the military systems. Most of the radars have frequencies beyond 1 GHz. So it has been obvious for us to introduce a frequency above 1 GHz in the scientific protocol. As summarized in Table 2.1 new communication technologies make use of frequencies above 1 GHz, for example 23 GHz for WiMAX, that is designed even for frequencies up to 60 GHz. Since a 9.70-GHz microwave generator was available in the laboratory at the time of the experimental design of this project, the frequency of 9.70 GHz has been integrated in the protocol of the study.

Also NATO has recently initiated research on the use of microwaves in a non-lethal weapon which can be used to deter and repel hostile elements. These microwaves are among others characterised by a frequency of 95 GHz; they are absorbed at a depth of 0.1 mm in the skin.

On the other hand new semiconductor solutions allow the use of higher transmission frequencies. All this makes it clear that man-made background noise at the higher microwave frequencies will continue to increase during the next few years.

2.2. Continuous Waves *vs.* Pulsed Waves: Indication of a Different Biological Effect?

Pulsed radiofrequency radiation nowadays is among the most ubiquitous of environmental electromagnetic factors. In the present text, the term "pulsed RF" or "pulsed waves" is used for any amplitude-modulated electromagnetic emission with carrier frequencies from units of megahertz up to 300 GHz, inclusive of an amplitude modulation in nothing or all like a typical radar emission pattern.

New radio technologies, *e.g.* UMTS and Bluetooth, like the variety of signal waveforms and modulation schemes used in wireless communications, have increased considerably in recent years. Most of those modern cellular systems operate in a pulsating mode by which the data (voice, image, and video) are transmitted in short pulses. Accordingly, interest in biological research to assess potential health risks of electromagnetic fields has grown. When we look in the literature, it is clear that parameters of microwave exposure are an important consideration in the production of biological effects: different durations of acute exposure lead to different biological effects and, consequently, different long-term effects occur after repeated exposure. In this context, the waveform of the electromagnetic field is also important, which can be demonstrated in differential effects that occur after exposure to pulsed *vs.* continuous wave microwaves. This will be treated in more detail in sections 2.2.1 – 2.2.3. Furthermore, the pattern of energy absorption in the body also contributes to the biological effect. Following the official standards, only the average power deposited in a small volume matters: the so-called SAR value [10]. However, there is a concern, based on some biological evidence, that the variations of the power may have a separate biological effect, and several international projects are underway to test this hypothesis. These findings raise the question of whether the whole-body average SAR may be used as the only determining factor in evaluating

biological effects of low-level microwaves. Other features of the exposure also need to be considered [11, 12].

While it is well known that pulsed fields can produce effects principally different from those of continuous wave [13, 14], potential implications of such effects for human safety and well-being continue to be debated and deserve by this more attention in future studies. Furthermore, it has been recommended that the public's concerns have to be taken seriously [15].

In this context, it has been shown by some studies that biological effects appear when the microwave carrier is amplitude modulated at an extremely low frequency (ELF) while they do not appear when the carrier is not modulated [16].

Moreover, our interest in taking into account possible biological effects from other than continuous waves has been increased by the extensive use of modern mobile communication systems together with the existing sources of pulsed microwaves like military and civilian radars. Indeed, ELF components are present in most digital mobile communication systems (GSM, DCS, DECT, TETRA, UMTS, WiFi, WiMAX), in digital audio broadcasting terrestrial (DAB-T) and analogue and digital video broadcasting terrestrial (DVB-T) systems. They are due to the time domain multiple access techniques, which offer the simultaneous connection of several users to a single base station. In TDMA techniques, a time frame is divided into timeslots and each timeslot is allocated to one user. The user's mobile unit transmits during its timeslot and does not during the rest of the frame. The base station transmits during a number of timeslots equal to the number of users in its area and does not transmit during the rest of the time. Moreover, some control signals define other time frames, so that the emissions are pulse-modulated at a frequency which is extremely low (from a few Hz to about 200 Hz).

The earliest suggestion that continuous wave and pulsed exposure might elicit different effects on animals is an anecdotal report by Boysen [17] that pulsed radiation was absorbed more strongly than was continuous wave radiation. Several early Russian experiments in which the effects of pulsed and continuous waves were compared are reviewed by Gordon [18]. In one experiment, the mean survival time of mice was more than tenfold greater following pulsed Very High Frequency (VHF) exposure than following continuous wave exposure at the same average power density. However, when an exposure at 3 GHz was used, lethality was higher and more rapid with pulsed waves. Former Soviet Union studies constitute an important source of information on (pulsed) radiofrequency biological effects. While many of these studies were flawed by lack of dosimetrical data, poor study design, a number of good-quality studies have demonstrated significant biological effects.

The effects of absorption of electromagnetic energy, when modulated, on brain tissue and cell membranes depend on the frequency and type of modulation. They appear to be especially important at frequency and amplitude modulation below 300 Hz, although few results are available which illustrate a definitive effect. These modulation frequencies are comparable to the electroencephalogram (EEG) wave frequency spectra. Electrical coupling of both frequencies is however unlikely [19]. Other studies mention biological effects on the brain activity induced by weak microwaves which are amplitude-modulated on the brain activity in rats [20].

It has been observed that rats acutely exposed during 45 minutes to pulsed 2.45 GHz (2-ms pulses, 500 pulses per second (pps), power density 1 mW/cm², average whole-body SAR 0.6 W/kg) showed retarded learning while performing in the radial-arm maze to obtain food rewards [21].

Deficits in memory functions, even transient ones, can lead to serious detrimental consequences.

Some studies in the literature do mention biological effects occurring when pulsed waves have been applied. Some of these effects are not demonstrated when using continuous waves. On the cellular level, the effects range from differences in calcium (Ca^{2+}) efflux, through interactions with cell membranes to changes in enzyme activity. There are among others a number of *in vivo* studies reporting on changes in brain wave pattern, alterations in behaviour of laboratory animals exposed to pulsed waves, and an occurrence of increased lymphomas in transgenic mice. The findings of a few of these studies have been shortly described and discussed from paragraph 2.2.1. on. There is, in the whole spectrum of the scientific literature, at least for each study mentioning a positive outcome another study mentioning no effect at all. That is the scientific reality up to now. In some cases, not enough data exist to be used as basis of an explanation of the observed responses, or even to establish firmly the nature of the response. Sometimes when duplication has been attempted, the original results were not confirmed. However, these experiments require a high degree of precision in engineering aspects of the experimental design, and lack of such precision could be a reason for the lack of reproducibility. Many of the studies that claim modulation-specific effects have not been replicated independently, but a lot of them also not disproved. This finding was important to us by establishing the protocol of our experimental study, in a sense that we considered to take into account both continuous waves and the so called pulsed waves at the same carrier frequency.

2.2.1. More in Detail: the Ca^{2+} Efflux

In a series of experiments on neuronal cells and heart cells starting in the middle of the seventies [22] and ending in the nineties [23], the Ca^{2+} efflux

technique has been applied. These investigations are based on the observation of Bawin *et al.* [24] that the waves of electroencephalograms (EEG) of cats can be influenced by RF fields of 147 MHz which are amplitude modulated in the frequency range of the spontaneous EEG waves of 5-20 Hz. Therefore Ca^{2+} efflux studies were carried out in the presence of these fields mainly on different neuronal cell preparations like chicken brain [25-30], cat brain [31], human neuroblastoma cells [32, 33]. In these investigations, different carrier frequencies of 50, 147, 450 and 915 MHz were applied and independently of the carrier frequency a change in Ca^{2+} efflux was detected, if the fields were sinusoidally amplitude modulated at a frequency of 16 Hz (frequency window). Interestingly, some of these studies did also show frequency windows around 50 Hz [27, 30, 33]. Even ELF fields alone have been reported to enhance Ca^{2+} efflux from chick brain in specific intensity regions [34].

Positive findings in Ca^{2+} efflux recordings on neuronal cells or tissues have mainly been demonstrated by three groups (Adey, Blackman, Dutta). Other groups (Shelton, Merritt, Albert) failed to confirm these findings [35-37]. Nevertheless, the three groups published a remarkable number of studies showing an increase or decrease in Ca^{2+} efflux due to acute exposure to RF fields sinusoidally amplitude modulated at 16 Hz. The results have been interpreted as a change in the binding of calcium to the external surface of the cell membrane. Inconsistencies in the results between the various studies have been attributed to differences in the applied temperatures [38].

Besides the investigations on neuronal tissue and cells, also one study on whole frog hearts was performed with the same technique at a carrier frequency of 240 MHz. Again, a sinusoidal amplitude modulation of 16 Hz caused an increase in efflux, effective SAR-values were 0.15 and 0.3 W/kg [39]. In a follow-up study on frog heart atrial strips this finding could not be reproduced although a wide variety of SAR-values was tested, ranging from

0.0032 till 1.6 mW/kg [40]. However, in the second study a different carrier frequency of 1 GHz and a different setup was used. Frog-heart atrial strips were employed instead of whole hearts. The authors attribute the differences between both studies to these variations.

The experiment by Tamburello *et al.* [41] points towards a difference between the biological effect of pulsed waves in comparison to continuous waves on isolated chicken embryo hearts. Heartbeat stimulation and the control effect of microwaves on the electrical activity of the heart have been analysed. The hearts were exposed to low-power, pulse-modulated microwaves at 2.45 GHz, 10 mW peak power, and 10% duty cycle. The estimated incident peak power density was 3 mW/cm². The repetition frequency was within normal physiological limits between 1 and 3 Hz. Before being exposed, the heart rhythm was rather irregular. When microwaves with a pulse repetition rate of 2.4 Hz were tuned on, the heart beat increased likewise until, above 2.65 Hz, the heart came back to beat irregularly. Hence, the heartbeat was synchronized with the signal within normal physiological ranges. This phenomenon is explained by an effect of pulsed modulation of the source on currents due to the calcium ions. Continuous wave exposure at the same peak power as the experiments with pulse modulation does not show any significant modification of the heartbeat. As the authors have pointed out, this suggests a low-thermal effect induced by pulse-modulated microwaves. The temperature of the sample is lower than at CW excitation; therefore, no heartbeat increase can be related to temperature variations [42].

2.2.2. More in Detail: Ornithine Decarboxylase (ODC)

An enzyme of the cytoplasm, which has been reported to be sensitive to RF fields, is the ornithine decarboxylase (ODC). ODC is a marker enzyme for DeoxyriboNucleic Acid (DNA) synthesis and thus cell proliferation. Byus *et*

al. [43] showed an increased ODC activity in three cell lines after one hour exposure to a field of 450 MHz sinusoidally modulated at 16 Hz at a power density of 1 mW/cm². This is in agreement with findings of Penafiel *et al.* [44], who also reported an increase in ODC activity in fibroblasts due to the exposure in an 835 MHz field at SAR-values between 1 and 3 W/kg. The ODC activity rose, only if the field was sinusoidally modulated at 16 or 50 Hz. Also pulsed RF fields according to the TDMA standard are able to induce the activity of the ODC [44]. The induction of ODC activity by pulsed or modulated RF fields can be mitigated if extremely low frequency noise is superimposed onto the signals [45] or if the fields are not modulated [46].

2.2.3. More in Detail: Interactions with Membranes

The cell membrane has been suggested as a likely site for the interaction with radio frequency fields [47]. A number of investigations have been performed concerning the question whether ionic current through cell membranes may be affected by the acute presence of RF fields. These studies have been carried out on both intact cells and isolated membranes, so called bilayer preparations.

Baranski *et al.* [48] reported an increased potassium (K^+) efflux in rabbit erythrocytes exposed to 3 GHz for up to 3 hours at power densities of 1 mW/cm². Using thermal controls heated in a water bath to an identical temperature as the exposed cells were unable to replicate these effects. Cleary and co-workers [49] observed an increased K^+ ion release - relative to thermal controls at around 24°C - by rabbit erythrocytes after CW or PW exposure to 8.42 GHz, for 2 hours at up to 90 W/kg.

Field *et al.* [50] found a small influence on the frequency of action potentials of snail neurons under very well controlled conditions. This was caused by a

field of 2.45 GHz pulsed at 100 Hz, pulse duration 10 μs and an SAR of 81.5 W/kg. The absorbed energy is relatively high and therefore most of the effects may be explained by a temperature rise. In the study on heart Seaman and DeHaan [51] showed decreases in the beating frequency of the heart, which cannot be explained by heating (2.45 GHz pulsed with 16 Hz, SAR 1.2-12 W/kg). In a study on frog heart Pakhomov *et al.* [52] failed to show an influence of pulsed 885 MHz and 915 MHz fields at SAR values below heating on heart rate. In a second study the same group recorded the force of contraction applying the same fields. During normal contractions they did detect a change in the contraction due to the applied fields.

There are some effects like the change in Ca^{2+} efflux and the elevation of ODC activity, which show sensitivity to modulated RF fields. In case of lymphocyte proliferation, pulsed fields proved to be more effective. Szerska *et al.* [53] studied the effect of 2.45 GHz fields continuous and pulsed wave between 100 and 1000 Hz, pulse duration 1 μs, on the proliferation of lymphocytes. The lymphocytes had been stimulated with the mitogen Phytohaemagglutinin (PHA) and stayed for five days in the field. Pulsed fields induced an increased proliferation of the cells at an SAR of 1 W/kg. Continuous wave fields did increase proliferation only if the absorbed energy was high enough to increase temperature.

Some of these studies might indicate that biological cells react in a different manner to pulsed electromagnetic fields compared to continuous wave fields. This is one reason why we have introduced in our scientific protocol both modes at the same carrier frequency. Some other studies investigating the difference between both exposure modes are cited in section 2.3 together with the obtained results.

The investigations presented here above might point to common mechanisms by which radiofrequency fields may act onto cells. However, one should be

aware that to date such a mechanism has not been formulated. Taken together, experiments applying modulated RF fields have been performed. If they revealed positive results, most have found frequency and power windows. A frequency window around 16 Hz was a common finding in most studies. A theory explaining these windows is still missing.

2.3. Thermal, Athermal, Non-Thermal, Microthermal, and Isothermal Effects of Radiation

Although the omnipresence of mobile phones is a fairly recent phenomenon, a lot of research has been performed about the biological effects of microwave radiation, especially the thermal effects, using a wide range of animal models, frequencies, and modulations. It is evident from these data that many of the reported effects result from either a rise in tissue or body temperature of about 1°C or more, or in physiological and behavioural responses for minimizing the total heat load [54]. These thermal responses have generally been used as part of the scientific bases for formulating restrictions on human exposure to microwaves [55].

Nevertheless, the possibility of non-thermal effects cannot be dismissed.

Data about the effects of microwaves form a heterogeneous ensemble of facts that are not readily classified in terms of thermal *versus* non-thermal interactions [56]. The first category includes interactions with the peripheral nervous system and certain neurovegetative functions, alterations in electro-encephalogram, changes in animal behaviour and, possibly, the permeability of the blood-brain barrier. The second category could include membrane interactions that affect the ion fluxes, the modulation of neuronal impulse activity, and possibly, induced arrhythmia in isolated hearts. Also included in this category could be the somewhat confused collection of dystonias and

behavioural effects that are often referred to as the microwave syndrome. For some time a number of individuals have reported a variety of health problems that they relate to exposure to electromagnetic fields. While some individuals report mild symptoms and react by avoiding the fields as best they can, others are so severely affected that they cease work and change their entire lifestyle. This reputed sensitivity to electromagnetic fields has been recently termed "electromagnetic hypersensitivity" (EHS). EHS is characterized by a variety of non-specific symptoms, which afflicted individuals attribute to exposure to electromagnetic fields. The symptoms most commonly experienced include dermatological symptoms (redness, tingling, and burning sensations) as well as neurasthenic and vegetative symptoms (fatigue, tiredness, concentration difficulties, dizziness, nausea, heart palpitation, and digestive disturbances). The collection of symptoms is not part of any recognized syndrome [57].

These problems may well be due to environmental factors unrelated to microwave exposure, but a possible non-thermal mechanism cannot be completely ruled out [58].

It is true that the growth and development in personal mobile communications has generated much public concern and scientific debate about the possible detrimental effects posed by exposure to low-level microwave fields. Particular concern has been expressed that low-level exposure, as that emanating from the cellular phone's base station, may increase among others the risk of cancer, impair the normal immunological functions, and influence the working of the central nervous system [59].

Bawin [22] suggested that the exposure to low-level microwave radiations could cause biological effects other than thermal provided they are amplitude modulated at a low frequency. A significant contribution to the field of bioelectromagnetics has been made by the research performed in the

former Soviet Union. Unfortunately, most of this research was published in Russian; these publications are scarcely available in the West. Even some key findings, which may affect the conceptual understanding of interaction mechanisms and approaches to RF safety, seem to be not known in the West, and their replication on Western laboratories has never been attempted. In 1971 Michaelson [60] mentioned that the importance of the difference between the Soviet and Western views is readily apparent when it is realized that practical consideration of maximum permissible exposure is based on the acceptance or rejection of non-thermal effects of microwaves as biologically significant.

Thermal effects of microwaves are well understood and are the only officially established biological effects until today. The World Health Organization (WHO) encourages further research efforts focussed on the so called athermal or non-thermal effects together with the possible biological effects after long-term microwave exposure [61].

Sheppard [62] suggested a categorization by which radio frequency studies are divided into three groups, *i.e.* “thermal”, “athermal” and “non-thermal”, according to the magnitude of the SAR and the actual or potential temperature change of the biological system. Thermal animal studies are those where thermoregulation is challenged, requiring that the animal responds by changes in perspiration, breathing, blood flow and related physiological measures in order to reduce body temperature. Thermal studies in animals generally require more than 4 W/kg and may result in body temperature increases of 2 to 5 °C. If exposures are so low that a significant temperature rise would not occur even in the absence of special cooling devices, exposures can be called “non-thermal”, but if temperature is held constant by removal of heat that would otherwise cause significant temperature increase, exposures can be designated as “athermal” or better “isothermal”. In other words, athermal/isothermal animal exposures, with

SARs in a range for 0.1 to 1 W/kg may elicit mild thermoregulatory responses, but body temperature remains unchanged. Non-thermal animal exposures, below an SAR of 0.1 W/kg, produce neither an effect on body temperature nor a thermoregulatory challenge.

Though, these terms have different meanings to different investigators. In 1997, Vander Vorst proposed to avoid the words "athermal" and "non-thermal", because these are negative definitions, and to consider the possibility of "microthermal" effects [63]. These could be related to a "trigger" action of microwaves, with the possibility of having a large biological effect produced by a small microwave trigger [64]. He considers that "isothermal" is to be used instead of "non-thermal" because the word isothermal is a precise definition. To evaluate operation at constant temperature, however, thermodynamics has to be used as an extra tool.

Biophysically, “non-thermal” effects are not defined by the degree of heat production, but rather by the mechanism of interaction. In that sense, a mechanism is considered “non-thermal” if the effect is caused directly by the interaction of the electrical or magnetic vector of the electromagnetic field with charges or dipoles of the tissue, but not by heating or activation of the thermoregulation system. An effect that is biophysically spoken classified as “non-thermal” should cause biological effects without the involvement of heat energy. Purely physically considered, this is not possible, because when microwave energy is deposited in tissue, the mechanism of energy transfer will create heat. The other meaning is that an effect takes place without an apparent change in temperature in the exposed system. In this case, subtle temperature effects are possible meaning that they activate common reactions of the biological system to a very low and probably only locally detectable degree of heating, without causing a temperature rise of the entire system. It may not be practical to make a distinction between these effects because of the difficulty to eliminating thermal effects. Pragmatically, we

propose to use the term “low-thermal” effects or “low-thermal” exposure conditions, which is based on officially established documents like the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. An experiment done at or below that level should be considered as “low-thermal”. For example, the ICNIRP guideline for general population exposure at 1 GHz is 5 W/m², which is corresponding with a basic restriction level of 0.08 W/kg including a safety factor of 50. The level at which effects should occur would be 4 W/kg. Any exposure below 0.08 W/kg would be considered as “low-thermal”.

As there will be explained in section 3.5, we have taken the ICNIRP reference levels as a basis for the determination of an athermal *i.e.* low-thermal exposure level for our experiments, because of the fact that between 100 kHz and 10 GHz, ICNIRP basic restrictions on SAR are provided to prevent whole-body heat stress and excessive localized tissue heating.

We prefer the term “low-thermal” or “microthermal” above “athermal” in this context, because the absence of any rise in temperature can never totally be excluded.

Over the past decade, various studies have reported that low-level pulsed or amplitude-modulated radiation can exert specific biological effects under some circumstances: the most noteworthy of these studies are also considered here [65-69].

Although the time variation of the power (or SAR) belongs to the realm of possible non-thermal effects and is thus outside the accepted guidelines, it is still of interest to understand the spectrum of the power. The case of high peak powers at low repetition frequencies resulting in low SAR’s may induce biological effects.

In the context of the practical significance of the SAR it is worthwhile to mention the *in vivo* experiment of Kues and Monahan [70]. They investigated the effects of pulsed microwaves on the eyes of monkeys. Exposures were at 2.45 GHz, impulse width 10 microseconds, 100 pulses per second at an average incident power density of 100 W/m² (SAR: 2.65 W/kg) on three consecutive days for four hours every day. Damage to the corneal endothelium and an increase in iris vascular permeability were found persisting for more than 72 hours of exposures. This report suggests that pulse-amplitude modulated exposure might have significantly lower thresholds for damage than continuous wave exposure, especially high peak power exposure with low SAR resulting from a sufficiently low repetition rate. Johnson *et al.* [71] also exposed monkeys to 1.25 GHz microwaves, pulse width of 0.5 microseconds, 16 pulses per second at an average ocular SAR of 3.5 to 4.0 W/kg, four-hour exposures, seven days a week for several weeks. At this high-peak, low repetition rate exposure, retinal damage was found, suggesting cone photoreceptor damage. Creighton *et al.* [72] have also found evidence of an enhanced effect of pulsed over continuous microwave exposure at equivalent average power level by observing cataractogenesis in the rat lens *in vitro*.

Another well-known example is the microwave hearing effect, where thermoelastic expansion of soft tissue in the head, as a consequence of exposure to very high peak power short pulses (like in military radars), has been proposed as the mechanism [73]. The resultant acoustic pressure wave travelling to the cochlea, and detected by the hair cells, may be perceived as an audible noise (typically a clicking or buzzing noise). It has been shown that although a high rate of instantaneous energy absorption is required to produce the auditory effect, the minuscule, but rapid temperature rise obtained from a single pulse, is below 10^{-4} °C. Tissue cooling is a slowly varying function of time and becomes appreciable only for times greater than

milliseconds [73]. Such auditory response is produced primarily by the energy delivered within approximately the first 50 μs of the leading edge of each successive microwave pulse. Where the ambient noise level is low, the minimum energy to produce a perceptible auditory effect is quite small. The threshold of perception is about 100-400 mJ/m² for pulses of duration less than 30 μs at 2.45 GHz, corresponding to a specific absorption of 4-16 mJ/kg. In the frequency range of 0.3 GHz to several GHz, the threshold specific absorption of 4-16 mJ/kg for producing the auditory effect, for 30-μs pulses, corresponds to peak SAR values of 130-520 W/kg in the brain.

The auditory effect depends on the energy in a single pulse and not on average power density. Guy *et al.* [74] found that the threshold for RF induced hearing of pulsed 2.45 GHz exposure (pulse repetition rate 3 Hz, pulse width 1-32 μs, peak power density 1250 mW/cm², average power density 0.1 mW/cm²) was related to an energy density of 400 mJ/m² per pulse, a calculated peak absorbed energy density per pulse of 16 mJ/kg, or energy absorption per pulse of 16 μJ/g, regardless of the peak power of the pulse or the pulse width (less than 32 μs); calculations showed that each pulse at this energy density would increase tissue temperature by about 5×10^{-6} °C. This is in accordance with the findings of Lin [75] who demonstrated that the auditory effect can arise at an incident energy density threshold of 400 mJ/m² for a single, 10 μs pulse of 2.45 GHz microwave energy, incident on the head of a human subject. It has been shown to occur at an SAR threshold of 1.6 kW/kg for a single 10 μs pulse of 2.45 GHz. The microwave hearing effect is the most sensitive biological effect induced by microwave radiation. The lowest threshold value expressed in units of average incident power density is 0.001 mW/cm² (peak power density 225 mW/cm², pulse width 10 μs, energy density per pulse 23 mJ/m²) for a 3 GHz exposure [76]; this value was due to the low pulse repetition rate of only 0.5 Hz, because for a given peak power, average power density depends

on the pulse repetition rate. Chou *et al.* [77] established the threshold of incident energy density per pulse at 15-30 mJ/m² for auditory perception of RF pulses in rats.

2.4. Blood-Brain Barrier

The nervous system is functionally composed of neurons (nerve cells) and supporting cells called glia cells. The nervous system can be subdivided into two major parts: the central nervous system (brain and spinal cord), and the peripheral nervous system which consists of 12 pairs of cranial nerves arising from the brain and 31 pairs of spinal nerves arising from the spinal cord and their associated ganglia. The brain lies in the cranial cavity and is continuous with the spinal cord. The integration of information takes place in the central nervous system [78].

The term blood-brain barrier was first introduced about a century ago by Paul Ehrlich to account for the observation that Prussian blue dye injected to the blood circulation failed to stain brain tissues [79].

2.4.1. Structure of the Blood-Brain Barrier

The mammalian brain is protected from potentially harmful compounds in the blood by the so-called blood-brain barrier (BBB). The BBB is of essential importance for the normal functioning of the central nervous system. The brain, composed out of billions of neurons, relies on synaptic transmission and summation enabling complex neural integration and coordination. Neurons need a stable fluid environment essential for their proper synaptic functioning.

The BBB is a selectively permeable, hydrophobic barrier that is readily crossed by small, lipophilic molecules. It is not permeable to hydrophilic (polar) and large molecules (molecular weight > 500 dalton (Da)).

Carbohydrates and proteins for example are excluded from the brain by the BBB. The selective passage restricts not only entry of toxic polar molecules and many drugs and therapeutic agents into the brain, but it is also a regulatory system that stabilizes and optimizes the fluid environment of the brain's intracellular compartment within certain narrow limits that are essential for life. A dysfunctioning BBB allows influx of normally excluded hydrophilic molecules into brain tissue. This might led to cerebral oedema, increased intracranial pressure, and, in the worst case, irreversible brain damage [80].

The BBB is an anatomic and physiologic complex associated with the cerebral microvasculature. The endothelial cells that line brain capillaries are cemented together by intracellular tight junctions (*zonulae occludens*). Anatomically, the BBB is further composed of a continuous basement membrane (basal lamina) surrounding the endothelial cells of the capillary. A network of astrocytic foot processes (astrocytic pseudopodia) envelops the tight junctions of the vascular endothelium and adheres to the outer surface of the capillary wall. This can be seen in Figures 2.4 and 2.5. [81].

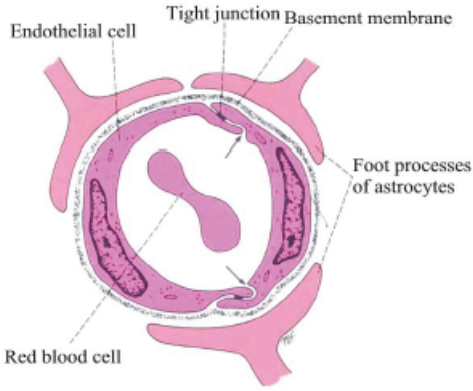

Figure 2.4: Cross-section of the blood-brain barrier with from inside the lumen to the outside: the endothelial cells, the basement membrane and the astrocytic foot processes.

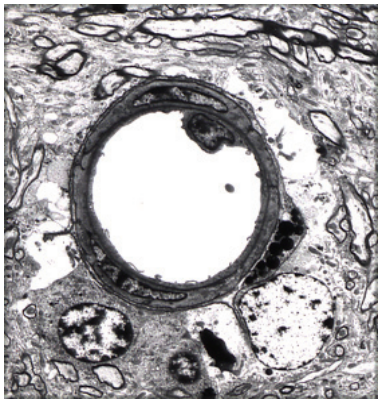

Figure 2.5: Electronic microscope image: brain capillary with surrounding astrocytic process.

As shown in Figure 2.6, more extensive tight junctions, absence of fenestrations, high mitochondrial density and sparse pinocytic vesicular transport are the hallmarks of brain capillary endothelial cells in contrast with endothelial cells in the rest of the body. In addition, pericytes encircle endothelial cells, providing structural support and aiding in controlling blood flow [82].

The BBB is bidirectional, able to exclude and transport materials either from the central nervous system or from the blood [83].

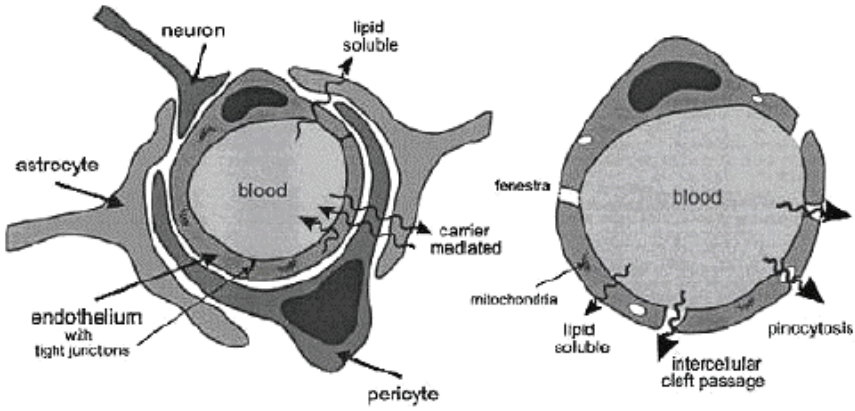

Figure 2.6: Schematic comparison between brain capillary (left) and general capillary (right).

The BBB cannot be absolute because the brain is dependent on the blood to deliver metabolic substrates and remove metabolic wastes. Therefore, the BBB must facilitate the exchange of selected solutes. Figure 2.7 shows how lipophilic substances, such as fatty acids, oxygen (O_2), and carbon dioxide (CO_2), readily cross the lipid bilayer membranes of the endothelial cell.

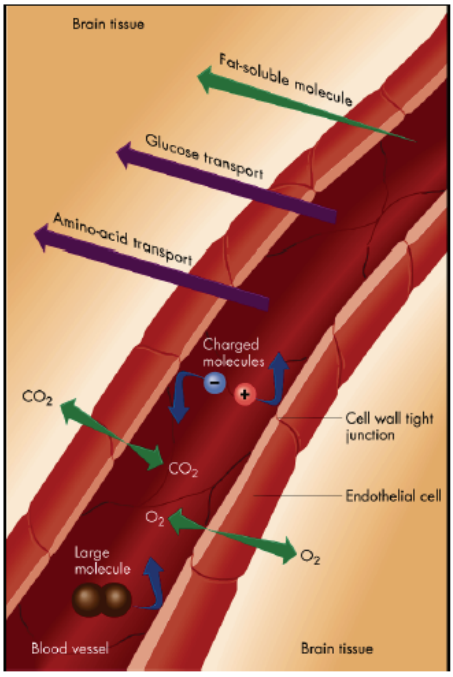

Figure 2.7: Transport of various substances across the BBB [85].

Although lipophilic molecules, glucose, ketone bodies and amino acids can enter the brain quite easily, most important metabolic substances are too polar to enter the brain efficiently by simple diffusion. For these compounds, carrier-mediated transport systems are present to facilitate their brain uptake (Figure 2.8.) [84].

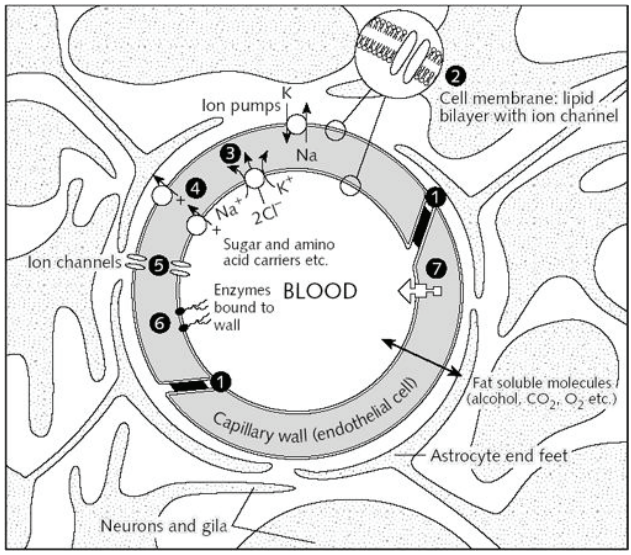

Figure 2.8: Diagram of a cerebral capillary enclosed in astrocyte foot processes. The essential characteristics of the blood-brain barrier are indicated: (1) tight junctions that seal the pathway between the capillary (endothelial) cells; (2) the lipid nature of the cell membranes of the capillary wall which makes it a barrier to water-soluble molecules; (3), (4), and (5) represent some of the carriers and ion channels; (6) the 'enzymatic barrier' that removes molecules from the blood; (7) the efflux pumps which extrude lipophilic molecules that have crossed into the cells.

The existence of carrier-mediated transport systems which operate across the BBB can be deduced on the basis of the selectivity that results from the binding of the solute to a recognition site on the carrier protein. Thus, carrier-mediated transport is saturable because the number of binding sites is limited. It is stereospecific because a specific structural arrangement of the solute is required for it to be recognized by the carrier; it can be competitively inhibited by structurally related compounds that compete for binding to the same carrier or noncompetitively by compounds that bind elsewhere on the carrier protein [86].

Several brain structures lack a BBB. These areas are adjacent to the ventricles and are called the circumventricular organs. They are involved in

the hormonal regulation of other organ systems and therefore must be able to respond to changes in the concentration of peptides and other substances in the blood. The circumventricular organs include the choroid plexuses, neurophysis, pineal gland and some other structures. Blood vessels in these areas of the brain have fenestrations which permit diffusion of blood-borne molecules across the vessel walls [87]. These areas of the brain are responsible for the regulation of the autonomic nervous system.

2.4.2. The Influence of Microwave Exposure on the Integrity of the Blood-Brain Barrier

A number of investigations on BBB disruption at a very low level of microwave exposure has captured increasing attention. Unintentional opening of the BBB may subject the central nervous system to assault from extraneous micro-organisms and substances which are toxic to the brain cells. About 40 scientific studies all over the world on the effect of microwave radiation on BBB permeability were reported until 2006 [88].

Among these studies, the reports showing increased BBB permeability after microwave exposure are about equal in number with those that do not mention changes in BBB permeability at high as well as low SARs. Some of the apparent discrepancies can be undoubtedly attributed to the complexity of the BBB and to differences in microwave exposure conditions, such as frequency, power level, and SAR distribution, and from differences in the use of a variety of assays and procedures to detect changes in BBB permeability [89].

The essay methods utilised include visual dye markers, such as Evans blue, rhodamine-ferritin, and sodium fluorescin. Evans blue is the most commonly used visible marker and is normally excluded from the brain. As it completely binds with serum albumin it provides a clear indication of any

dye-albumin penetration into the brain. On the other hand, gross and ultrastructure observations of horse radish peroxidise in brain slices can be used to evaluate the blood-brain integrity. Radioactive tracers and electron microscopy are alternative methods to assess permeability changes in the BBB. As in normal physiological conditions albumin (molecular weight 69 kDa) is excluded from the brain, BBB disruption can be traced in brain slices by histopathological detection of any extravasated endogenous albumin using for example goat anti-rat albumin as the primary monoclonal antibody [90]. The delivery of therapeutic agents, which normally do not have access to most brain tissues, is another indication of alteration in the permeability of the BBB [91].

The first investigations showed changes in BBB permeability at high SAR levels which caused an elevation in brain temperature to about 42 °C for substances normally excluded from the brain [92]. However, Frey *et al.* [93] already in 1975 evaluated at low power exposure the integrity of the BBB by comparing the fluorescein dye concentrations in brain slices in rats after 30 minute exposures to 1.3 GHz microwaves CW (24 W/m², 2.2 W/kg whole-body averaged SAR) or pulsed (10 W/m² average, 1 W/kg averaged SAR, 21 W/m² peak, 1000 pulses per second (pps), 0.5 ms pulse duration). The dye concentration in the brain slice was significantly higher in both CW or pulsed exposed rats than in sham-exposed animals. Pulsed microwaves induced a similar but more pronounced increase in dye concentration than CW microwaves. Oscar and Hawkins [94] also compared the efficacies of 20 minute exposures between CW and pulsed 1.3 GHz microwaves on the disruption of the BBB. They concluded that brain permeability was increased for small inert polar molecules, depending on molecular weight of the tracer, for mannitol (molecular weight = 182 Da) and inulin (molecular weight = 5 kDa) but not dextran (molecular weight = 60 kDa) in rats exposed to CW and pulsed microwaves at average power densities lower

than 30 W/m². Increased permeability was observed immediately and four hours later after exposure, but not 24 hours after exposure. They also found that, depending on the specific pulse characteristics used, pulsed microwaves could be more or less effective in altering the BBB permeability than CW of the same average power density. For example pulsed microwaves as 1000 pps, 0.5 µs pulse duration, duty cycle 5×10^{-4}, were less effective in increasing the brain mannitol uptake than CW of the same average power density. On the other hand, wider pulses with a lower repetition rate (5 pps, 10 µs pulse duration, 5×10^{-5}) were much more effective in increasing the brain mannitol uptake than CW.

More recent reports, like a series of reports from Lund, Sweden [95-97], suggest that microwave exposure can alter BBB permeability at SARs that are well below the maximal permissive level for cellular phone, for instance 1.6 W/kg, including extremely low levels, such as 0.016 W/kg. Figure 2.9 depicts a cross-section of the central parts of the brain of a sham-exposed rat (left) and a cellular phone-exposed rat (right); both slices are stained with albumin antibodies to reveal albumin as brownish spotty or more diffuse discolorations. In the left picture, albumin is visible in the central inferior parts of the brain (hypothalamus), which is a normal future. On the right side of Figure 2.9, albumin is visible in multiple small foci representing leakage from many brain vessels.

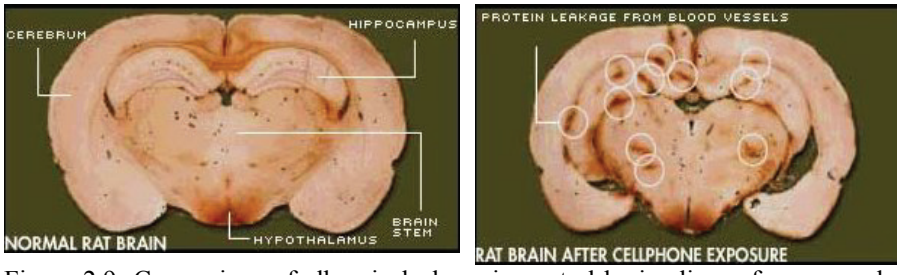

Figure 2.9: Comparison of albumin leakage in central brain slices of unexposed (left) and exposed rat (right) [98].

Fritze *et al.* [99] performed a similar study without confirming exactly the same results as the group from Lund: they found no serum albumin extravasation at SAR levels of 0.3 – 1.5 W/kg, but they did at a SAR of 7.5 W/kg. It has to be said that this study was not a replication study, because they used an entirely different exposure system than that of the Lund group. However, the Swedish results are duplicated in another laboratory, where plasma protein extravasation in rat brain and *dura mater* was observed (SAR 2 W/kg averaged over the brain) [100]. This is an interesting finding, because, although the blood vessels of the *dura mater* are devoid of tight junctions, permeabilization of these vessels to plasma proteins is involved in migraine mechanisms suspected to be activated by mobile phones [100].

A more recent publication of the Swedish mentioned, parallel with the albumin leakage from the brain vessels, occurrences of damaged neurons - the so-called 'dark' neurons - in the cortex, hippocampus, and basal ganglia in rat brains after exposure for two hours to GSM cell-phone fields of various strengths (SARs of 0.02 W/kg and 0.2 W/kg) up to 50 days following microwave exposure [98]. The 'dark neurons' (stained with cresyl violet) are abnormal neurons that appear as black and shrunken nerve cells, which are interpreted by the investigator as evidence for neuronal damage in microwave-exposed rats.

In a study of Tsurita et al. [101], immunostaining of serum albumin was used to study the effect of exposure to 1439 MHz TDMA cellular phone field on the permeability of the BBB in rats exposed for two or four weeks (2 W/kg peak SAR in rat brain, 0.25 W/kg average whole-body SAR). They found no significant changes in any of the groups. Results from Evans blue injection were also negative.

In addition to the effects found in animal studies, recent *in vitro* studies, using endothelial cell models of the BBB, suggest that exposure to mobile phone microwave radiation at the permissible level could increase BBB permeability significantly compared to unexposed samples [102, 103].

Any final conclusions as to the potential effect of mobile phone radiation on blood-brain barrier are premature. The presently available scientific evidence does not provide clear answer weather the opening of the BBB can be induced by microwave exposure of the general public to television, radio, mobile phone, WiFi and WiMAX transmitters. Additional studies are urgently needed to clarify this issue [104].

2.5. Summary

Based on today's radiofrequency environment where the cellular phone, DECT, WiFi and some radars (with their carrier frequency which is situated around 1 GHz) take an important place, we have selected among others the 970-MHz frequency to be incorporated in our scientific protocol, because it is situated around the frequently used 1-GHz frequency.

The 9.70-GHz frequency is the second frequency to be used in our experiments. The factor of 10 between both frequencies is a trump in our scaled model based on a physical-biological interaction mechanism. Besides, some military and civilian radars, and new wireless devices (WiMAX,…) are working with frequencies between 1 GHz and 10 GHz. The 9.70-GHz frequency has been chosen as a frequency representing those higher frequencies in the environment. An example of a new microwave application is the US active denial system which is intended to be used as a non-lethal weapon for crowd control purposes during military operations.

We have introduced two different modes, because of indications in the scientific literature that pulsed wave exposure might produce other biological effects than continuous waves do.

The entire design of the study is developed to focus on possible low-thermal effects, because thermal effects of microwaves are well known.

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Chapter 3 Description of the Experiment

3.1. Choice of Animals: the *Wistar Han* Albino Rat

While *in vitro* studies are important in determining the mechanisms of interaction and in identifying appropriate biological endpoints, they can not serve alone as a basis for health risk assessment in humans. Whole animal studies are necessary in order to evaluate the integrated response of various systems of the body. *In vivo* studies are closely related to real life situations. They often employ several different field levels and exposure periods to investigate dose-relationships and/or possible compensation mechanisms. Animal studies are used to develop biological principals applicable to human risk estimates. It is known that activated or developing physiological systems are in general more sensitive to noxious stimuli than static ones [1].

During the past decades, research in live sciences has been growing exponentially. The laboratory rat has long been used in experimental physiology and has made significant contributions to several complex areas of mammalian biology. Of all laboratory animals, the rat (*Rattus norvegicus*) is undoubtedly one of the most frequently used animals in biological and medical research and is probably the most investigated species on earth. Rats are extremely well studied animals, so we know an enormous amount about their basic biology. For example, the rat is a highly valuable model organism for the analysis of many complex areas of biomedicine such as cardiovascular disease, metabolic disorders, neurological disorders and behaviour, autoimmune diseases, cancer and renal diseases. The wealth of information and the multiplicity of strains available with different specific

characteristics make the laboratory rat an indispensable tool for biomedical research. The laboratory rat has been selectively bred to be docile.

Investigators must consider a wide variety of factors when selecting the best animal model for research. An obvious choice is the animal species and model used historically for the same type of research. Most research in bioelectromagnetics is carried out on rodents. Considerations in selecting an animal model depend on both scientific and practical issues.

The *Wistar* and, to a lesser extent, the Sprague-Dawley rat are the most popular rat strains for laboratory research. Most laboratory rat strains descend from a colony of rats established at the *Wistar* Institute in 1906. *Wistar* rats are an outbred strain of albino rats belonging to the species *Rattus norvegicus* and is notably the first rat strain developed to serve as a model organism at a time when laboratories primarily used *Mus musculus*, or the common house mouse. The *Wistar* rat is characterized by its wide head, long ears, and having a tail length that is always less than its body length.

The appropriateness of the system for testing the proposed hypothesis, the responses of the animal to the blood sampling procedure and experimental handling are important factors to be evaluated in the process of choosing the best fitted animal model.

The number of animals needed must be based on sound statistical design. Statistical power calculations should also be considered when evaluating sample sizes. In this context, it could be demonstrated that one needs at least 20 rats per group to be able to take statistically significant conclusions.

The small size of the rat (about 30 cm long and 8 cm high) is an advantage for handling and housing. Besides, the 30 cm length corresponds well with the wavelength of one of the two incorporated frequencies, namely the 970 MHz. The importance of resonancy phenomenon in considering

biological effects of electromagnetic fields has been explained in section 2.1.2.

The availability of the rat, including appropriate age, sex and numbers is no problem. The availability of facilities and care for housing together with the right equipment and expertise are present at the *Université catholique de Louvain* (UCL). Proper housing and management of animal facilities are essential to animal well-being, to the quality of research data and testing programs in which animals are used, and to the health and safety of personnel. A good management program provides the environment, housing, and care that permit animals to grow, mature, and maintain good health. It provides for their well-being and minimizes variations that can affect research results.

Animals should be housed with a goal of maximizing species-specific behaviours and minimizing stress-induced behaviours. For social species, like the rat, this normally requires housing in compatible pairs or groups.

Rats from a specialised breeder company are delivered with a certificate providing the investigators with information on the animals' genetic background, diet, health status, vaccinations and other characteristics so that variables which might confuse the research results are minimized. This constitutes another advantage in comparison to human epidemiological studies.

Human beings are very complicated creatures, but our cells contain the same fundamental materials as those of all living things. Rats are genetically (for 90%) and physiologically similar to humans. They are vulnerable to more than 200 of the same diseases. If selected carefully, they have narrow genetic variations and thus demonstrate little biological variation in response to stimuli. At approximately 2.75 billion base pairs, the rat genome is smaller than the human genome, which is 2.9 billion base pairs. However, the rat

genome contains about the same number of genes as the human genome. Furthermore, almost all human genes known to be associated with diseases have counterparts in the rat genome and appear highly conserved through mammalian evolution, confirming that the rat is an excellent model for many areas of medical research [2].

Computer modelling can reduce study time and the number of animals needed by simulating biochemical cascades and their outcomes. Computers can not, however, simulate how the circulatory system for example will respond to the incoming electromagnetic field. Nor can they come close to simulating the complex physiology of the billion of cells in an animal.

For all these reasons, we have chosen for the rat as animal to be used in our experiments. Due to his size, ready availability, and within defined strains, relative uniformity, rats are the preferred laboratory animals for the proposed study. Furthermore, rats are an obvious choice as they are strong, relatively inexpensive, easy to handle, they require limited storing space and are low on maintenance costs.

Their relative short life span (2.5 to 3 years) makes it possible to study disease development over a much shorter period of time in comparison to human beings. It also enables us to expose the rat for about 70% of its life time starting from the beginning of maturity. The use of human subjects is ethically not feasible nor desirable nor practical, because these studies would involve studying humans throughout their lifetimes. Epidemiological studies performed with humans are generally biased by variables which the investigator can not control. Besides, in the field of cancer epidemiology, the long latency periods (which can reach 30 or 40 years) mean that the exposures that must be considered are those that took place in the past, sometimes several decades before the onset of the disease.

3.2. An Innovating Concept in Animal Epidemiological Studies

Rats used in biomedical research are typically reared in small cages that lack key features of their natural environment. These conditions impose constraints on behaviour, brain development and stress [3]. Findings in animal psychology research learn that narrow housing causes an enormous stress response in the rat [4]. This stressor creates a supplementary variable, which is susceptible to mask secretion of certain corticosteroids [5], 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 adrenocorticotropic hormone (ACTH) can increase up to the 5-fold of non restrained rats [6]. In this context the effect of restraint induced stress was recently reported by Stagg *et al.* [7]. The observed levels of ACTH and corticosterone increased by nearly 10-fold 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 [4, 5]. 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 all together 124 rats. This will be described in more detail in section 3.3.

Observing freely moving animals gives supplementary information on the influence of exposure on behaviour 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. Because of 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 and 6 months [8].

It is important that the rats are all initially strange to the cage or the exposure unit, and that they are introduced at the same time. Even an interval of ten minutes between one male and the next may lead to conflict; evidently, a male introduced early establishes itself very quickly as a resident. Provided these conditions are satisfied, all male colonies of rats may be maintained indefinitely. In a settled colony, a serious clash between males is unusual, even in a quite dense laboratory population.

At the age of about three months, rats display a marked set of changes. This is the period of loosely called “puberty”.

The rats are accustomed to the handlings during experimental procedures, one month before the start of our experiment. Each day, during two hours, the rats are placed into the exposure units to familiarize them with the experimental conditions and with the presence of the experimenters. Daily

gently handling of rats results in animals that are less vulnerable to stress [9, 10].

3.3. Unambiguous Rat Marking

As we decided to collectively expose rats in an exposure unit suitable for at least 40 freely moving rats, in stead of confining them in narrow housings during exposure, a supplementary practical problem raises in identifying instantaneously the rats. Moreover, our investigation concerns a long-term exposure of rats during 21 months at which a simple felt-tip pen mark – standard used in short-term experiments – is not efficient anymore because of the moult and the frequently licking of the fur as part of the daily hygienic activity of the rats. Therefore, pen marks stays readable for only two till three days before they completely have disappeared in one week, whereas hair clipping may last for two weeks. For an analogous reason tail marking is not a solution to this problem either. Tail and toe clipping are not recommended whereas ear punch identification could not be utilized because it may be obliterated by contact between rats. To identify the rats during the whole experiment in general and during blood sampling in particular, an unmistakable permanent identification method to distinguish one rat from another has been thought out. The ear of the rat is pierced following a formerly established pattern of figures. Ten metal substrates with needles in the shape from zero till nine are placed in the holder of a pair of tongs where after the pattern with needles is pierced in the ear. Immediately after, permanent ink is rubbed into the piercing, leaving a permanent ear mark. This procedure is carried out under total anaesthesia for one minute on the basis of sevoflurane. An example of a typical unambiguous rat marking can be seen in Figure 3.1.

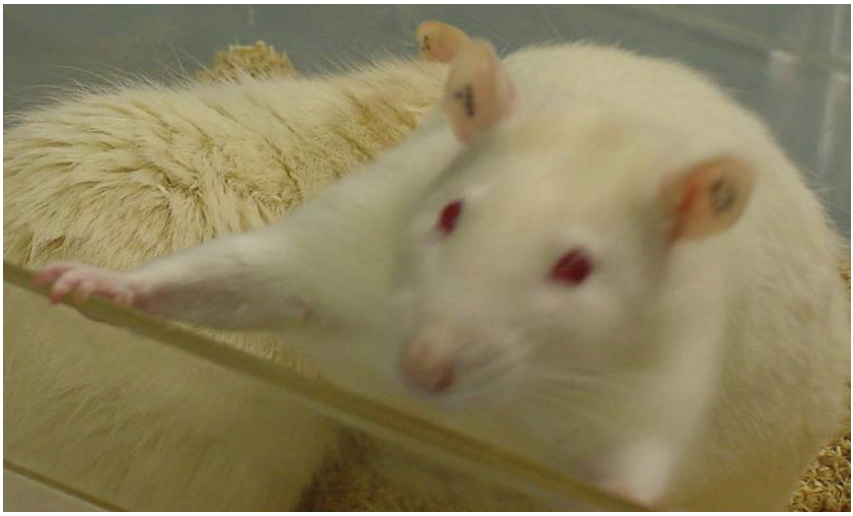

Figure 3.1: Permanent unambiguous way of rat marking.

3.4. Microwave Exposure System

Collective exposure of rats to a high frequency electromagnetic field at low-thermal conditions is rare to be described in literature. To our knowledge, no scientific study about such large scale group exposure of rats to microwaves for more than one year has been published in recent years.

The entire microwave exposure system has been designed for simultaneous exposure of four groups of rats, the sham-exposed included. This concurrence of exposures is an important element in the light of the statistical power of the experiment, because a similar *modus operandi* excludes any differentiated influence of the time component regarding the four groups. In this setting, the only variable is definitely the exposure characteristic (frequency and mode) to which the rats are exposed. The exposure system is composed of four exposure units, each for one exposure type (*i.e.*, 970-MHz continuous wave, 970-MHz pulsed-amplitude-modulated, 9.70-GHz continuous wave, sham-exposed group). We have not

included a 9.70-GHz PW exposure in the scientific protocol because of the practical reason that a sufficient powerful generator which could deliver a peak power equal to ten times the mean power output of the CW exposure system (see also section 3.5) was not at our disposal in the planning phase of the experiment.

Amplitude modulation (AM) constitutes a time varying change in the field strength of a microwave carrier. Pulse-amplitude modulation is a special type of AM that is often used in RF digital communication systems. Pulse modulation often involves 100% amplitude modulation of the RF carrier. Here, the carrier signal is turned on and off completely during the pulse cycle, what gives it the characteristic of a typical radar signal. Figure 3.2 shows a typical pattern of the signal we used, termed as the 970-MHz pulse-amplitude modulated exposure, practically abbreviated as 970-MHz pulsed wave (PW).

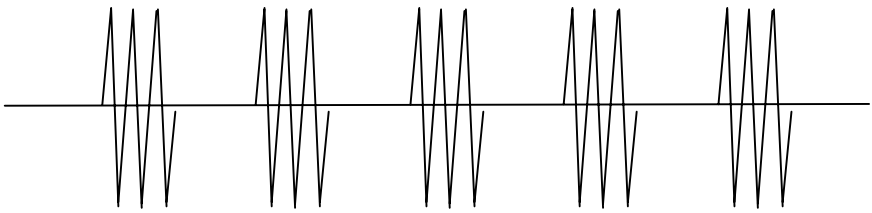

Figure 3.2: Typical pattern of the 970-MHz PW exposure.

Each exposure unit is built around an identical basic part which is composed of a custom made box of polyethylene (1.25 m x 0.70 m x 1.20 m) and a cover which can entirely shut the exposure unit. The only different part between the four exposure units is the transmitting facility which is designed in relation to the respective frequency to which the rats will be exposed.

The general scheme for the three 'radiating' exposure units is as follows: a microwave generator is connected to an amplifier which is connected to the entrance of the respective antenna. The antenna is on top of the exposure unit with the rats at the bottom, in the far-field region of the antenna.

The polyethylene box, with walls of 0.04 m thickness, is covered at the outside with radar absorbing material, itself covered with wood (multiplex 18 mm) to prevent any artefacts arising from objects placed into the microwave field. Each unit is easily suitable for 40 freely moving rats.

The cover of the box, with an antenna on top, is also self-constructed. A typical example can be seen in Figure 3.3. A neon lamp is incorporated in the cover. It is important that the light exposure of the rats is not interrupted during the experiment, because the blood sampling protocol includes the determination of light sensitive hormones: the corticosterone and adrenocorticotropic hormone release follow a circadian rhythm. A fan is integrated in the cover to improve the internal ventilation of the exposure unit. The apertures in which fit the neon lamp and the fan are covered by fine-meshed metal gauze to shield the interior of the exposure unit from background noise if such should be the case, so that the entire exposure unit is electromagnetically isolated from the outer environment. It has to be mentioned that the laboratory and the animal facility room are located in the basement with ceilings made out of armoured concrete. This is an excellent shield for incoming electromagnetic fields which are blocked totally. It is for example not possible to make a phone call by cellular phone, because no wireless connection with an outside base station is possible for that reason. In this framework, the background electric field strength has been measured and we recorded a background level that was inferior to the sensitivity of our electric field probe.

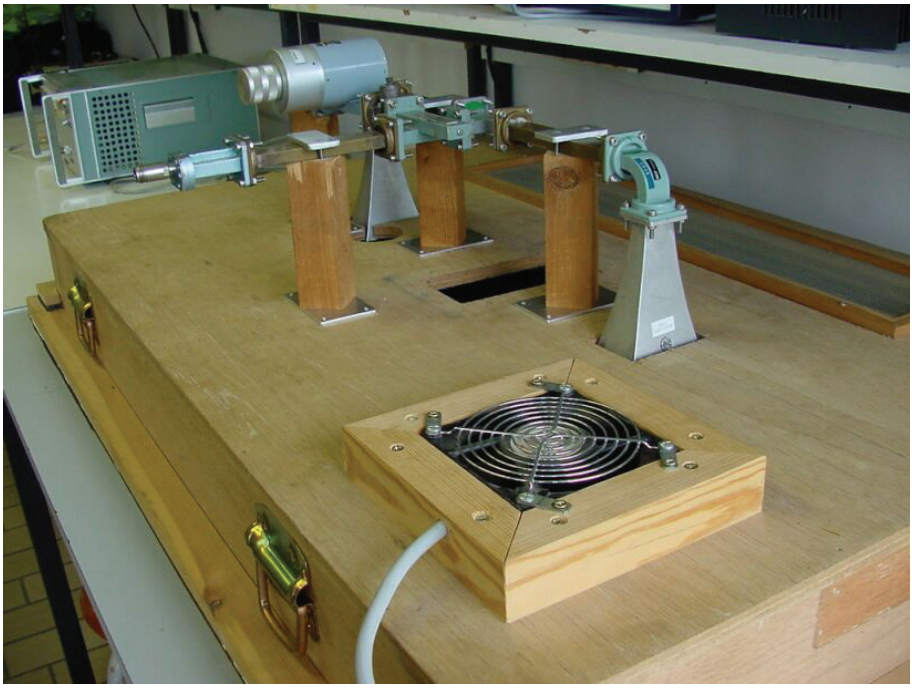

Figure 3.3: Detail of the cover of the 9.70-GHz CW exposure unit with two horn antennas.

The height of the cover (0.23 m) is such that the rats are exposed in the far field of the antenna. Indeed, the region in the immediate neighbourhood of the antenna aperture is the near field. It extends several antenna diameters from the aperture. The Fresnel region is the intermediate area between the near field and the far field. In the Fresnel region, rays from the radiating aperture to the observation point are not parallel and the antenna radiation pattern is not constant with distance. The farthest region from the aperture is the Fraunhofer or far-field region.

In the Fraunhofer region, the radiating source and the observation point are at a sufficiently large distance from each other so that the rays originating from the aperture may be considered parallel to one another at the

observation point. At distances from the source larger than $2D^2/\lambda$ (where D is the largest characteristic dimension of the radiating antenna and λ being the wave length) and in the absence of obstacles, the electric and magnetic field may typically be at right angles to one another and perpendicular to the direction of propagation of the electromagnetic wave (a transverse electromagnetic (TEM) mode exists). Also in the far field the ratio of the electric to the magnetic field strength has a constant value (termed the intrinsic impedance of free space which equals to 377 Ω). The boundary R between Fresnel and Fraunhofer regions is usually taken to be $R = 2D^2/\lambda$.

In the near field, the maxima and minima of the electric and magnetic fields do not occur at the same points along the direction of propagation as they do in the far-field region of the antenna. The electromagnetic field structure may be highly inhomogeneous and typically there may be substantial variations from the plane wave impedance for free space of 377 Ω ($= 120\pi$). As a consequence, in some regions almost pure electric fields may exist, and in other regions, almost pure magnetic fields. They are often non propagating in nature. Objects located near sources may strongly affect the nature of the fields. For example, placing a measuring probe in the near field of an antenna may change the characteristics of the fields considerably. Field strengths in the near field are more difficult to characterize, because both electric and magnetic fields must be measured and because the field patterns are complicated; the power density tends to vary inversely with R in stead of R^2 (as in the far field), and may display interference patterns.

In the far field only the electric field strength or the magnetic field strength needs to be measured, because when one of them is known, the other can be easily calculated.

Besides, since the power density P (W/m²) is the vector cross-product of the electric field strength E (V/m) and the magnetic field strength H (A/m), the following far-field relationship can be derived:

$$P = \frac{E^2}{377} = H^2 \cdot 377 \qquad (3.1)$$

The power density is the magnitude of the Poynting vector which is described by the following equation [11]:

$$\overline{P} = \overline{E} \times \overline{H} \qquad (3.2)$$

with $\overline{P}$ the Poynting vector, $\overline{E}$ the electric field vector, and $\overline{H}$ the magnetic field vector. The magnitude of the Poynting vector represents the rate at which energy flows through a unit surface area perpendicular to the direction of wave propagation .

From above, it may be clear that measurements and calculations are easier in far-field conditions than in the near field. For this reason, we have constructed our collective exposure units in a way that the rats are exposed in the far field of the antenna.

Both the 970-MHz exposure units are equipped with a Kathrein GSM antenna with the following dimensions: 0.325 m x 0.265 m x 0.050 m. The antenna is characterized by a gain of 9 dB and a 3-dB beamwidth in the horizontal and vertical plane of 65° and 70°, respectively. It can be quickly calculated that the Fraunhofer region begins at a distance of 0.684 m from the antenna. It is thus clear that the rats are located in the far field of the antenna.

The 970-MHz continuous wave is generated by a HP 8620 Sweep Oscillator, coupled to a microwave amplifier 3W EIN, Model 603 L. The power at the end of the amplifier is 1.2 W.

A second exposure unit exposes another group of 31 rats to a 970-MHz pulse-amplitude-modulated signal, with a pulse repetition frequency of 1 Hz and a duty cycle of 10%. The central part of the pulse generator is composed of a voltage controlled oscillator (ROS-1700 W, Mini Circuits) - fixed at 970 MHz with a power output of 6.05 mW - and a Switch MSW-2-20 of Mini Circuits who cuts the continuous wave signal of the voltage control oscillator, leading it to the Motorola 30 W EGSM amplifier (30 dB, 920-990 MHz band width) every 0.1 s. The LM 555 timer is a highly stable device for generating accurate time delays or oscillation. For working as an oscillator, the free running frequency and duty cycle are accurately controlled with two external resistors and one capacitor. The amplifier is powered by an Advance TWE 1200 H27 supply and generates an output signal of 12.6 W to the antenna by a coaxial cable. For the rest, the exposure unit has an identical design as this of the 970-MHz CW exposure unit, including previsions for ideal interior light conditions and air-evacuating devices.

The 9.70-GHz continuous wave exposure unit is equipped with two horn antennas with the following dimensions: 0.0725 m x 0.0500 m x 0.1000 m. Applying the above formula learns that the far field begins at a distance of 0.317 m from the antenna.

The exposure part of the 9.70-GHz CW unit is composed of two X-band 16-dB horn antennas which are integrated in the cover of the exposure unit with an interdistance of 0.5 m. Both identical antennas have a 3-dB-beamwidth of 8° and 30° in the E-plane and the H-plane, respectively. Two horn antennas are used in order to illuminate the entire bottom of the exposure unit. The low power (9 mW) microwave signal is generated by a gunn diode (oscillator type 6068 of MI), amplified by a 6W FW-5 Power Amplifier (bandwidth 4 GHz – 12 GHz, 30 dB gain) to the level of 2.8 W, and delivered at the entrance of the antennas where finally a power of

1.75 W is delivered. This value is continuously monitored with a power meter HP 432 A. The present power level fits well with the idea of a low-power (300-500 μW/cm²) exposure of the rats inside the exposure unit.

The last group of 31 rats is sham-exposed: they enter every day in an identical exposure unit, but the only different variable is the absence of any exposure.

Each exposure system is designed in a way that the E-vector is orientated in the horizontal plane in which the rats are freely moving.

The rats are exposed in this exposure system (Figure 3.4) two hours a day, seven days a week during 21 months.

Figure 3.4: Collective exposure system for freely moving rats.

3.5. Determination of an Appropriate Exposure Level

The dosimetric concept was initially developed for and successfully applied to protection from ionizing radiation. The ionizing standard is based on an established correlation between the dose and the biological effects, whereby the dose is defined as the energy absorbed per unit mass. Derived values, such as the incident radiation in terms of radiometric quantities, and the definition of dosimetric terms, such as whole body and organ dose, population dose, and relative biological effectiveness, were also defined.

Although this is quite a straightforward approach for protection from ionizing radiation, it is much less suitable for non-ionizing radiation. The reason is that the interaction path between exposure, dose and biological effects depends on many more parameters than ionizing radiation. Hence, several further additions were required to make the dosimetric concept applicable to frequencies below 300 GHz.

In the microwave frequency range all current standards are based on the premise that the underlying mechanism, which correlates well with the biological endpoint, is the temperature increase in the tissue caused by the absorption of non-ionizing energy, *i.e* thermal effects. At the frequency range of 40 MHz to 6 GHz, the electromagnetic field penetrates deeply into tissue, causing an increase in the random molecular motion. Since the body's capacity to detect temperature increases at greater tissue depth is lower than at the skin, the exposure cannot directly be compared to that of infrared or light exposure, which is absorbed within the first few millimetres of the skin tissue.

One of the difficulties in developing a safety standard was that a straightforward definition of a dose which correlates well with heating effects is not possible. The reason is that whole-body or local temperature

increases not only depend upon the amount of energy absorbed and the effects of passive heat dissipation but also to a very large extent upon complex thermoregulatory processes in the body. Since those processes depend upon a number of parameters (*e.g.* specific organs, environment, health status, etc.), it is not the initial temperature increase in that tissue that is used to define the dose but the power absorbed per unit mass (W/kg), called SAR.

In the article where the ICNIRP guidelines are presented [12] is mentioned that many laboratory studies with rodents and non-human primate models have demonstrated the broad range of tissue damage resulting either from partial-body or whole-body heating producing temperature rises in excess of 1 to 2 °C. The sensitivity of various types of tissue to thermal damage varies widely, but the threshold for irreversible effects in even the most sensitive tissues is greater than 4 W/kg under normal environmental conditions. Established biological and health effects in the frequency range from 10 MHz to a few GHz are consistent with responses to a body temperature rise of more than 1 °C. This level of temperature increase results from exposure of individuals to a whole-body SAR of approximately 4 W/kg for about 30 minutes. Available experimental evidence indicates that the exposure of resting humans for approximately 30 minutes to electromagnetic fields produces a whole-body SAR of between 1 and 4 W/kg resulting in a body temperature increase of less than 1 °C [13]. Animal data indicate a threshold for behavioural responses in the same SAR range [14]. Exposure to more intense fields producing SAR values in excess of 4 W/kg can overwhelm the thermoregulatory capacity of the body and produce harmful levels of tissue heating.

These data form the basis for an occupational exposure restriction of 0.4 W/kg, which takes into account a safety factor of 10 for other limiting conditions such as high ambient temperature, humidity or level of physical

activity. An additional safety factor of 5 is introduced for exposure of the public, giving an average whole-body SAR limit of 0.08 W/kg. For the general public, which includes sensitive subpopulations, such as children and elderly, a basic-restriction level of the SAR at 0.08 W/kg would provide a further margin of safety against adverse thermal effects from radio frequency fields. As the basic restrictions are expressed in quantities that are internal to the body and that therefore can not be measured practically in the body of a human being, reference levels, expressed in measurable units of power density (W/m^2) or electric field strength (V/m), have been introduced. These levels are provided for practical exposure assessment purposes to determine whether the basic restrictions are likely to be exceed. The reference levels are expressed in quantities that are measured in the absence of human beings.

As the ICNIRP guidelines – based on thermal detrimental effects – guarantee the occurrence of no adverse health effects at exposure levels equal or lower than the established basic restrictions and reference levels, we have taken these values as a basis for our experiments on low-level effects of microwaves. Following this line of thought, it is reasonable to assume that there will be no harmful effects due to temperature rise in any biological tissue when not exceeding these exposure levels. This is an excellent starting point for fixing an appropriate exposure level in view of our investigation concerning the possible “low-thermal” effects of microwaves.

After careful examination, we decided in our epidemiological study to scale the frequency with the size of the animal. When extrapolating the results of, for instance, animal experiments to human exposure, the conditions of electromagnetic similitude can be applied [15]. These conditions are often used in a reduced form termed “frequency scaling”. They enable us to use results obtained with a given object and adapt them to predict the results to be obtained with another object, similar in form to the first and differing

only by a scale factor. This yields a precisely calculated value for the exposure level to be used at any frequency. This is indeed original. The audiences to which we have presented the planned experiment and partial results [16-18] found this very interesting.

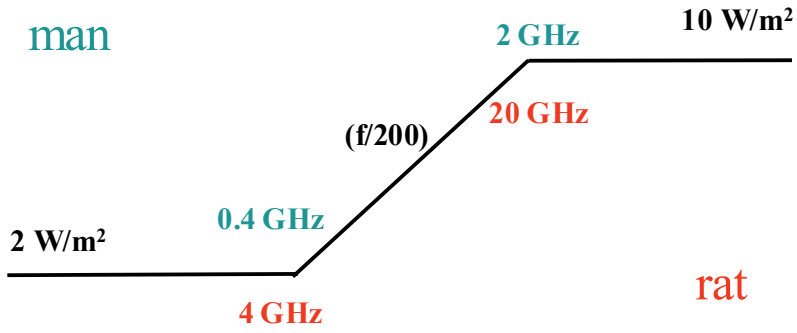

Figure 3.4: ICNIRP basis for man, adapted to rat.

Assuming that the ratio in size between a human and a rat equals 10, we have adapted the ICNIRP reference levels for the general public exposure, to the size of the rat by multiplying by a factor 10 the key frequencies (Figure 3.4), given that the reference levels are based on thermal effects essentially generated as a consequence of a resonance effect because of the compliance between wavelength and characteristic dimension of the body in question. This means that the exposure of a rat at 970 MHz corresponds in terms of resonance phenomenon to a human exposure of 97 MHz. The basic ICNIRP reference value for man at 97 MHz is 2 W/m². Thus a power density of 2 W/m² or 200 µW/cm² is a suitable power density for the rats to be exposed to at 970 MHz. Following an identical reasoning leads us to the fact that a 970-MHz exposure for man corresponds to a 9.70-GHz exposure for rats. ICNIRP guidelines stipulate for a frequency of 970 MHz a reference

level for man, expressed in power density, of f/200 (f = frequency), *i.e.* 970 MHz/200 or 4.85 W/m² or 485 μW/cm². This exposure level is chosen for the rats at 9.70 GHz. The corresponding SAR value is 0.08 W/kg and is to be considered as low-thermal: the ICNIRP basic restrictions are based on acceptable thermal effects, below which no adverse effects occur.

The exposure level of the 970-MHz pulse amplitude-modulated group is derived from that regarding the 970-MHz CW group, dividing this latter by the duty cycle of 10%, *i.e.* multiplying it by a factor 10. The basic idea behind, is to achieve the same average exposure level in the 970-MHz PW exposure unit as in the 970-MHz CW exposure unit.

The frequency-scaling application is well known. It is often said that the conditions of electromagnetic similitude are satisfied when a smaller size model is submitted to a correspondingly smaller wavelength, that is, a correspondingly higher frequency. From this, it is frequently understood that a ten-time smaller model will yield the same results as the original one if the frequency is the tenfold. It must be emphasized that the SAR distributions will only be similar in the two bodies, not equal, and that the equivalence is strictly valid for no losses. We are aware that there are obviously losses in living tissues. Losses, hence microwave heating, are certainly not correctly taken into account, while lossless field effects might well be correctly taken into account.

As literature data show no clear agreement on the power density related to the “low-thermal” threshold, some experiments with different dosage levels were performed by a few investigators. Taking into account that a power density of 100 W/m² was found by Jensh [19] as a borderline for low-thermal exposure of rats at 915 MHz, we may be sure that the exposure level in our experiments (200 μW/cm² at 970 MHz), derived from the ICNIRP guidelines, has to be classified as “low-thermal”. A confirmation of the

plausibility of our approach can be found in the findings of J. Streckert *et al.* [20] who demonstrated that below a power density of 65 W/m² no increase in body temperature could be identified. Hence, they conclude that the thermoregulation process of the *Wistar* rats works effectively up to power densities of 65 W/m² at 900 MHz. Body temperature was recorded using rectal thermocouples. Finally, they calculated that a power density of 60 W/m² in their exposure system was equivalent to an average whole-body SAR of 2.2 W/kg, which is a mean value for the different postures and positions of the rats in their cages. This data can be used to derive an approximation of the SAR in our experiment, where a power density of 200 µW/cm² (2 W/m²) in the absence of rats was recorded at the bottom of the exposure unit. Applying the ratio 2/60 to the SAR of 2.2 W/kg leads to a whole-body SAR of 0.073 W/kg relating to our rats. This matches extremely well with the SAR value of 0.08 W/kg to which our rats are exposed and which we had first theoretically derived from the ICNIRP guidelines as explained before. Durney *et al*. [21] analytically calculated whole-body average SAR of a medium rat (320 grams) for E-polarization at an incident phone-wave power density of 1 mW/cm². It can be seen in Figure 2.2 that in the resonance frequency region the whole-body average SAR for rat is 0.6 W/kg. Comparing the incident power density of 1 mW/cm² to the power density applied in our experiment, *i.e.* 0.2 mW/cm², yields a ratio of 5. Multiplying the whole-body average SAR of rat at resonance frequency (0.6 W/kg) by 1/5 leads to an SAR related to our exposure level of 0.12 W/kg, maximally. Moreover, Figure 2.2 shows at 970 MHz - one of the exposure frequencies used in our experiments – an SAR for rat equal to 0.3 – 0.4 W/kg (incident power density 1 mW/m²). Taking into account the same factor of 1/5, an SAR of 0.06 – 0.08 W/kg can be derived related to the exposure level in our experiments. This calculated value is consistant with the experimentally obtained values formerly mentioned in this text. From all the data and scientific input cited above, it must be clear that the exposure

level to which the rats in our experiment are exposed has to be considered as low-thermal. Besides, during a trial exposure session, no change in rectal temperature between exposed and sham-exposed rats could be detected.

3.6. Dosimetry Measurements in the Exposure Units

A well-established dosimetry of the incoming electric field in nearly 80 sampling points inside each unit, in absence of the rats, was carried out before the start of the experiment. The evaluation of the field uniformity is carried out by means of an electric field measurement, using the Isotropic Field Monitor (FM 2000) of the company Amplifier Research. The omnidirectional probe is connected to the accompanying reading device, which displays the electric field strength by a fibre optic cable avoiding any interaction with the incoming electromagnetic field. Afterwards, the corresponding power density values were derived from these data. The bottom of the exposure unit is first subdivided in 77 squares of 10 cm². In a following step, the probe is placed successively in the centre of each square where the electric field is recorded. This procedure is repeated consecutively for the 970-MHz CW, the 970-MHz PW and the 9.70-GHz CW exposure units. The mean value in rounded figures of the electric field strength in the 970-MHz CW exposure unit, in the absence of rats, is 28 V/m; the mean value of the electric field strength for the 970-MHz PW and the 9.70-GHz CW exposure unit is 91 V/m and 33 V/m, respectively. This corresponds to the following power densities expressed in round figures: 200 µW/cm², 2200 µW/cm² (corresponding mean power density = peak power of 2200 µW/cm² x duty cycle 10% = 220 µW/cm²) and 300 µW/cm² in relation to the 970-MHz CW, 970-MHz PW and 9.70-GHz CW exposure, respectively.

The measurements sessions revealing the amplitude of the electric field strength and corresponding power densities learn that the practical obtained values match very well with those that we had put foreword theoretically.

These are based on the ICNIRP guidelines as explained in section 3.5, *i.e.* 200 µW/cm² and 485 µW/cm² regarding the 970-MHz and 9.70-GHz exposure, respectively.

A graphic representation of the 970-MHz CW electromagnetic field is shown in Figure 3.5. The compilation of the plots gives a good indication of the uniformity of the electromagnetic field; it learns that the overall electric field strength deviation is 30%, which can be considered as the best practical achievable distribution.

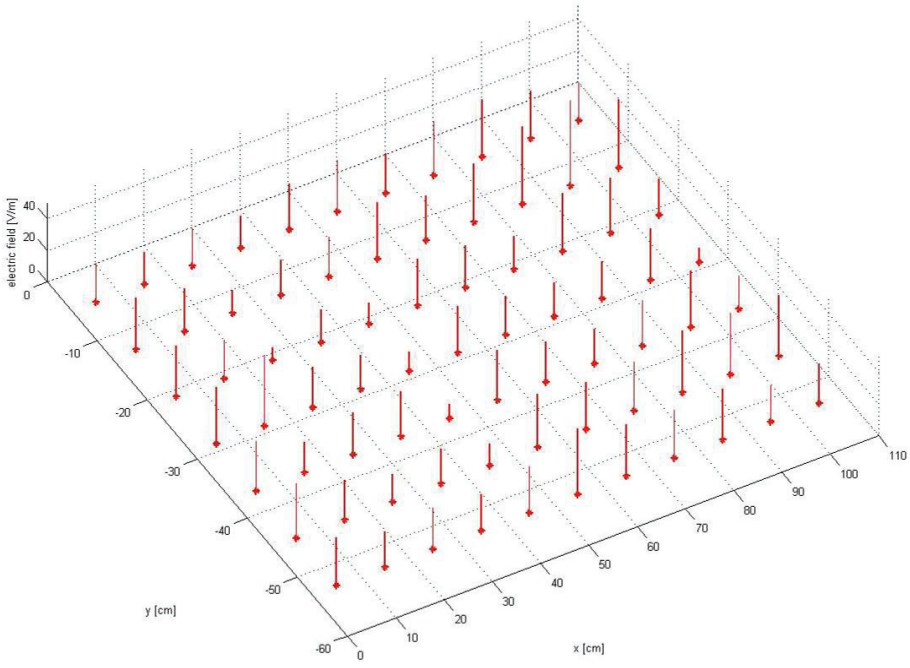

Figure 3.5: Spatial representation of the electric field in the 970-MHz CW exposure unit.

The result regarding the measurement session on the electric field strength at the inside of the 9.70-GHz exposure unit, *i.e.* 312 µW/cm², exactly represents 62% of the assumed value. By studying the spatial distribution of the electromagnetic field inside the exposure unit, it is clear that the low electric field strength at the outer contour is responsible for this non-achievement of the 500 µW/cm² power density goal. This can be observed in Figure 3.6.

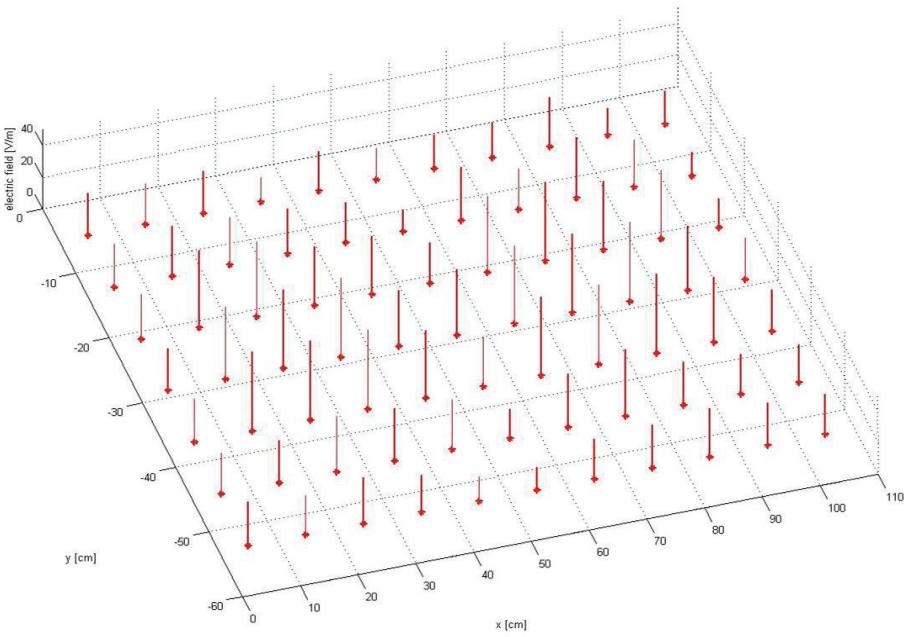

Figure 3.6: Spatial distribution of the electromagnetic field at 9.70 GHz CW inside the exposure unit.

This rather low value for the recorded power density can be explained by the characteristics of the exposure system. The 3-dB-beamwidth of the horn antenna (28° and 30° in the E-plane and H-plane, respectively) is a little too small to illuminate the entire bottom of the 9.70-GHz CW exposure unit.

At the moment of the practical realization of the exposure unit, there was no alternative at our disposal.

When one considers the interior exposure place as being the area minus a 10 cm contour along the four walls of the box, the mean power density is 422 µW/cm² (standard deviation 106 µW/cm²), being 84 % of the required theoretical value. Based on daily observations during exposure, we can state that in practice, the majority of the rats are in the centre of the exposure unit. Only a few rats are laying against the wall of the exposure unit. This can be avoided by placing pvc platforms (height 12 cm) in these outer regions with a power density less than 150 µW/cm².

3.7. Sequence of Operations

Young male *Wistar* albino rats, 12 weeks old, 200-230 grams, were obtained from a commercial source (Charles River Laboratories). A veterinary certificate stating the good health of the animals was presented by delivery. The rats have been tested on parvoviridae, paramyxoviridae, coronaviridae, bunyaviridae, reoviridae, gastro-intestinal helminths, amoebidae, dorylaimoridae, eucoccidioridae, trichomonadidae, and ectoparasites.

During the entire experiment, the health status of the rats has been followed up by a team of veterinarians, and blood punctures have periodically been taken to be sure that the animals are in good health.

All animals were housed in the same room of a conventional animal facility building with a 12 hours light/dark cycle with lights on from 07 a.m. to 07 p.m. at a temperature of 23 °C +/- 1°C and a relative humidity of 50% +/- 5%. The light intensity in the environment during day time was 60 lux. The ventilation rate of the room is 15 air volume changes per hour.

The rats are housed by two in a spacious polycarbonate/ Macrolon cage type IV, provided with a layer of sawdust (3/4; BMI, Helmond, The Netherlands) cage when they are not taking part at the experiment. The menu is composed of certified standard laboratory pelleted food (RMH-B; Hope Farms BV, Woerden, The Netherlands) specially prepared for rats. The animals have free access to the rodent chow and to tap water ad libitum. The tap water, approved for human consumption, has been extra analysed before the start of the experiment to be sure it is free of pathogens and chemical contamination. All experimental procedures performed on the animals are in accordance with the Guide for the Care and Use of Laboratory Animals and the protocol has been in writing approved by the bio-ethics committee of UCL on December 15th, 2003. The animal facility of UCL is fully accredited by the services of the Belgian Federal Government. Upon arrival, all rats were quarantined for two weeks. There has been provided a period of two weeks prior to the start of the experiments to allow them to acclimatize in the laboratory. The animals were familiarized with the exposure procedure and the daily handling by being placed in the exposure unit, but without switching on the exposure system.

Rats are subdivided in four groups of 31 animals at random upon arrival. We have foreseen a group of rats for each type of exposure, *i.e.* 970-MHz CW, 970-MHz PW, 9.70-GHz CW and the sham-exposed group. Daily, each group is introduced in its respective exposure unit for two hours, seven days a week, during 21 months. The different groups never come into contact with each other. After exposure, the rats are replaced in their spacious polycarbonate cages and each group is stored in a separate inox aluminium rack. Cage cards are utilized to identify the strain of rat, number, principal investigator and research protocol. Rats are observed clinically twice a day, morning and evening, throughout the experiment for possible signs of illness or behavioural alterations.

Blood samplings and analyses are carried out in a blind manner. Serial blood sampling is done every 3-4 months, six in total, starting with a base line bleeding session. Different trials to select the most appropriate blood sampling methods prior to the definitive sampling have been carried out. The blood sampling trials we performed, based on the several available methods, are described in section 3.9.

At the end of the experiment the rats are euthanatized by carbon dioxide for further anatomopathological study. They are conserved in a 10% solution of formaldehyde. A sample has already been analysed. The results can be found in the last part of this work.

3.8. Choice of Physiological Parameters

3.8.1. Selection of Haematological Parameters

Since we are one of the first laboratories having designed an exposure system susceptible to detect possible low-thermal effects, we could not find much information on selected parameters in previous low-thermal studies.

Moreover, we performed a long-term study, whereas most of the other experiments only considered exposure periods from a few days to a few weeks. The possibility of a cumulative effect from subthreshold exposures during the life time of a rat could be evaluated. Such an effect presumably could originate from clinically unapparent damage after short-term exposure which could manifest after prolonged exposure.

We have based our selection of physiological parameters to be investigated on scientific literature and practical feasibility. A general blood analysis based on haematology featuring the number of erythrocytes, leucocytes, reticulocytes, haematocrit, haemoglobin and its derived parameters as there

are Mean Corpuscular (cell) Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), is a general accepted standard for a first indication of possible biological effects from environmental influences on animals and men [22].

Erythrocytes or red blood cells are nonnucleated cells that transport oxygen and carbon dioxide. Their precursor cells in the red bone marrow, reticulocytes, usually develop into erythrocytes within one or two days after their release from red bone marrow [23]. Thrombocytes or blood platelets, nonnucleated as well, are responsible for coagulation and haemostasis. Leucocytes or white blood cells can be further subdivided into granulocytes, lymphocytes, and monocytes. The granulocytes are polynuclear and can be subdivided into basophils, eosinophils, and neutrophils, the last being in charge of the phagocytosis of pathogens. The function of lymphocytes is to identify antigens and produce antibodies. The monocytes play their part in the immune system by phagocyting antigens [24, 25].

The haematocrit (%) is a measure of red blood cell mass and is defined as the ratio of the volume of erythrocytes to the total blood volume.

The erythrocyte's red colour is due to the globular protein haemoglobin, which transports oxygen used in aerobic respiration. It is composed of four polypeptide chains and four iron-containing haem groups. It is the iron molecule at the centre of each haem group that binds oxygen [23]. The red blood cell count (RBC) measures the number of red blood cells present in the blood. The relationships between the haematocrit, the haemoglobin level, and the RBC are converted to red blood cell indices through mathematical formulas on which base different kinds of anaemia are classified. The indices include the measurements of mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration [26].

MCV is the index most often used. It measures the average volume of an erythrocyte by dividing the haematocrit by the RBC [27]. The MCV categorizes red blood cells by size and is used to classify anaemia. Normocytic anaemia is characterized by normal-sized cells and a normal MCV; microcytic anaemia is characterized by small cells and a decreased MCV while large cells and an increased MCV are typical indications for macrocytic anaemia [26].

The MCHC measures the average concentration of haemoglobin in an erythrocyte [27]. This index is calculated by dividing the haemoglobin by the haematocrit. The MCHC categorizes red blood cells according to their concentration of haemoglobin. Cells containing a lower than normal concentration of haemoglobin are hypochromic cells, in contrast to normochromic cells. Because there is a physical limit to the amount of haemoglobin that can fit in a cell, there is no hyperchromic category. Haemoglobin contains iron, which attributes blood its characteristic red colour. When examined under a microscope, normochromic erythrocytes stain pinkish red with a paler area in the centre. Hypochromic cells are lighter in colour with a larger pale area in the centre. Anaemia is categorized as hypochromic or normochromic according to the MCHC index [23].

The average weight of haemoglobin in an erythrocyte is measured by the MCH [27]. The formula for this index is the sum of the haemoglobin multiplied by 10 and divided by the RBC. MCH values usually rise or fall as the MCV is increased or decreased.

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 SAR. It is of interest evaluating those haematological parameters which have been reported in

prior studies as being altered by non-ionizing electromagnetic fields in our units 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 [28, 29]. However, there are a few studies revealing haematological effects at lower power densities too. Baranski [30] exposed guinea pigs and rabbits to 3 GHz, pulsed and continuous waves, 35 W/m², 3 hours a day for 3 months. Increases in absolute lymphocyte counts and no alterations in the granulocytes counts were observed. Goldoni [31] *et al.* reported that haematological examinations at a two year interval in occupationally exposed workers showed a statistically significant decrease in thrombocytes and leucocytes. Ray *et al.* [32] exposed rats 3 hours per day for 60 days at pulsed 7.5 GHz electromagnetic field (0.6 ms pulse width, average power level 0.6 mW/cm², SAR 0.03 W); they found significant changes in haematological parameters. Budinscak [33] 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 [34] described changes in erythrocytes and leucocytes exposed to microwaves at an incident power density of 13 mW/cm². Matausic and colleagues [35] exposed rats to 2.45 GHz at an average power density of 10 mW/cm², 2 hours a day, 5 days a week, during 30 days. Results revealed an insignificant increase in total erythrocytes. Total leucocyte count was significantly decreased for 8 days of exposure, while relative lymphocyte count was significantly decreased from the first day. Trosic *et al.* [1] studied the influence of 2.45 GHz microwave exposure, 5-10 mW/cm², whole-body average SAR 1-2 W/kg on cell response assessed by 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 haematological 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 [36].

3.8.2. Corticosterone and ACTH

Exposure to electromagnetic fields has been related to the occurrence of stress responses [37]. It is known that a significant increase in temperature can generate a stress response in animals. The endocrine responses to acute microwave exposure are generally consistent with the acute responses to non-specific stressors, such as heat, or with changes in metabolism caused by hyperthermia [38]. It has been reported in several papers that plasma corticosterone levels were significantly increased by exceeding thermal levels of microwave exposure; an increased (0.7-1.5 °C) colon temperature is needed to generate a significant effect [39]. The hypothalamus-pituitary-adrenal axis is one of the main mediators of the endocrine response to stress. When a source of stress is perceived by the central nervous system, neurotransmitters in the limbic system stimulate the hypothalamus to produce corticotrophin releasing factor. This reaches the anterior pituitary gland where it stimulates the release of ACTH. This hormone in turn is released into the blood circulation and stimulates the adrenal cortex to secrete glucocorticoids like corticosterone. Corticosterone is the most abundantly secreted glucocorticoid in the rat and helps the body to deal with stress. It acts as a negative feedback mechanism controlling the release of ACTH.

The function of glucocorticoid hormones is to prepare the body for physical activity by switching from anabolic to catabolic processes. Corticosterone and to a lesser extent, ACTH are released following a circadian rhythm with a peak hormone secretion towards the end of the light period. It is therefore obviously important to sample glucocorticoids at the same time of the day if

repeated measurements are to be made on different days. The dependency of microwave-induced secretion was demonstrated indirectly by absence of increased corticosterone concentration in hypophysectomised rats to microwaves [40]. In more recent papers investigators suggest that microwaves even at a low-thermal level could alter the stress level [41-43]. However, some of these investigations couldn't be confirmed by replication studies [44]. Exposure to low-level pulsed microwaves has been reported to affect brain neurochemistry in a manner broadly consistent with responses to stress. The acute exposure of rats to pulsed 2.45 GHz (2 ms pulses, whole-body average SAR 0.6 W/kg, SA 1.2 mJ/kg per pulse) was found to alter cholinergic activity in various regions of the forebrain [45]. Central cholinergic activity was increased after 20 minutes of exposure but decreased after 45 minutes, and repeated exposure engendered compensatory changes in the concentration of cholinergic receptors [46]. Stressors as acute restraint and noise can induce similar changes in central cholinergic responses, suggesting that low-level microwave exposure may be a source of mild stress. The similarity of the effects of microwaves and those of established sources of stress led to the speculation that microwave exposure is a stressor [47]. Other studies refer to so called hypersensitive people, suffering among others from stress caused by electromagnetic fields in the environment [48-50].

It is clear that thermal effects of microwaves are well known; though, our investigation is focussed on the low-thermal effects during a long period of exposure compared to the live span of a rat. Stress effects are known to cumulate over time and involve first adaptation and then eventual break down of homeostatic process when stress persists [42]. Taken together all these gathered data, we wanted to test the hypothesis as low-thermal microwaves could interfere with the basic stress level in rats. Of available knowledge in microwave induced stress, corticosterone provided the most

consistent endpoint to quantify the degree of stress past to microwave exposure. Stress in general leads to the activation of the pituitary-adrenal axis which produces an increase in both ACTH and corticosterone in blood plasma. Therefore, we selected two hormones involved with the stress response, *i.e.* corticosterone which is released by the adrenal cortex, and ACTH which is released by the pituitary gland in a negative feedback mechanism related to corticosterone. Both hormones are secreted following a circadian rhythm with a maximum at the beginning of darkness. Every 3 - 3.5 months, blood was taken from rats – each time at the same hour to cope with the circadian rhythm- and analysed to determine corticosterone and ACTH levels in the peripheral blood.

3.9. Blood Sampling

Blood can be sampled from animals using different techniques with differing impacts on the animal's discomfort due to differences in handling restraining, anaesthesia and the volume taken.

Criteria are among others: a rapid method allowing sampling 31 rats in approximately two hours, several (6) samplings in a life time, a possibility to withdraw 3 ml at once needed for analysis of the selected parameters (*cfr*. section 3.8). Good laboratory practice advice that a maximum of 10 % of the total blood volume can be sampled every two weeks (or 15 % of the total blood volume with a 30-day recovery period) taking into account that 7 % of the body weight is composed of blood [51]. Assuming that a typical 3-month old male rat has a body weight of 350 grams, the corresponding total blood volume is 24.5 ml from which 2.2 ml to 3.7 ml can be taken within a two weeks interval. A fourteen day recovery period is needed for the average healthy animal to recover from his blood loss. Although the blood volume is restored within 24 hours after blood withdrawal, two weeks is needed for all

constituents of the blood to return to normal. If less than the maximum amount of blood is taken, the animal will replace blood constituents at the rate of 1 ml/kg/day. Anaesthesia is used prior to blood collection from the orbital sinus (*cfr.* section 3.8) to restraint the rat and minimize distress to the animal and to the person performing the blood collection. Besides, this method of chemical restraining decreases the potential for complications due to injury to the eye. The animal is not returned to its cage until complete haemostasis has been achieved. Haemostasis is achieved using gauze and direct gently pressure.

Because of the circadian rhythm with which the stress hormones corticosterone and ACTH are secreted in the blood, we have a window of only a few hours to perform the sampling of 124 rats. So, we have been looking for a quick method for sampling 3 ml blood.

We tried blood sampling from the tail vein. The whole procedure to prepare the rat before the sampling was too time consuming, because the tail had to be placed in warm water for a few minutes to cause dilatation of the vein so that it became much more visible. It also took too much time to aspire the needed 3 ml of blood. This procedure could be carried out without anaesthesia, but the rat had to be immobilized. Furthermore, immobilizing conscious rats is very stressful to these animals and is therefore often used to study stress responses. In rats, it may even be more distressing than footshock stress of the same duration [52]. On the other hand, when anaesthetised anyway, the total time duration which anaesthetics were administered was much longer when collecting blood tail vein puncture than by retro-orbital sampling.

Retro-orbital puncture is frequently used to obtain blood from rats [53]. The blood sampling is performed while applying a short-acting anaesthetic to ease the rats. They were anaesthetized with a mixture of 8% sevoflurane

(*Sevorane*) and 92% medicinal oxygen (at a rate of 2 l/min) in a plexiglass induction box (25 cm x 15 cm x 15 cm). The rat was taken out of the induction box as soon as the palpebral reflex had disappeared. Between treatments, the box was cleaned with warm water and dried with a paper tissue. Orbital puncture was performed with a glass capillary and the blood was collected in an Eppendorf tube. If bleeding occurred (1% of the cases) after sampling, it was stopped by gently pressing a gauze pad on the eyeball after closing the eyelids. Immediately after this procedure, the rat was placed back in its home cage. Righting reflexes returned well after completing the blood collection by orbital punction; this was in a lesser extend the case for the two other blood sampling techniques.

The blood cell count is carried out by the use of the Advia 120 haematology analyzer from Bayer Diagnostics, which provides an automated analysis of the blood samples by counting and distinguishing different cell types. The white blood cell identification is based on cytochemical light scatter and light absorption measurements.

Blood is taken 6 times during the exposure period and the parameters taken into account are among others: white blood cells, erythrocytes, leucocytes, monocytes, haemoglobin, haematocrit, MCV, MCHC, granulocytes, ACTH and corticosterone.

3.10. Summary

We have chosen for an animal study in which the *Wistar* albino rat is used as animal to be exposed to microwaves, because the rat is one of the best studied laboratory animals. It is easy available, easy manipulating and doesn’t ask special facilities.

To reduce stress, the rats are collectively exposed in exposure units where they can freely move around. Before, the rats have been socialized to the laboratory environment, to the investigators and to each other. Because of the long-term characteristic of the study, we have thought out an unambiguous identifying system where the rat is given a tattoo in the ear. Blood sampling is performed six times during the whole exposure period.

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Part II Analysis of Blood Parameters

Chapter 1 Statistical Analysis

1.1. General Procedure

In our experimental setting, we are faced with animals exposed to four different conditions, *i.e.* four exposure types: 970-MHz CW, 970-MHz PW, 9.70-GHz CW and the sham-exposed group, which is the reference group. We are typically interested in whether the average performance under one condition, *i.e.* the sham-exposed one, differs significantly from that of another condition in the study. Unfortunately, simply looking at the means for each exposure will not be particularly informative because in almost all instances, these means will be numerically different. The issue facing us is whether the observed mean differences are statistically significant, that is, whether the differences are likely to recur or to be reliable if the study was to be repeatedly done. Statistically, we are asking if it is likely that the four conditions represent different populations or if the exposure has an effect on one or more dependent variables. To cope with the first part of the question - prior to the proper test for statistically evaluating the significance of the difference in means in all populations for a specific parameter - we performed the Levene test, which is a test of the equality of variances assumption. This test is explained in more detail hereafter in this section. Further on, the most commonly used procedures to compare group means are ANalysis Of Variance (ANOVA) [1] and Student's t-test [2].

The ANOVA tests whether our several populations of rats have the same mean value by comparing how far apart the sample means are with how

much variation there is within the samples. The ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Analysis of variance is used to test the hypothesis that several means are equal. In other words, the ANOVA specifies if there is somewhere a statistically significant difference for a specific parameter between at least two out of the four considered groups in our study.

Box plots visually show differences between groups. For each parameter, they represent graphically the distribution of its values. The boxes in the box plot represent middle quartiles, the horizontal line in the box indicates the median and the whiskers depict the range of the value for the considered parameter.

When comparing the means of *only two groups* in a study, for example the mean of a selected dependent variable in the sham-exposed group with its homologous in a selected exposed group, a Student t-test can be used [2, 3]. The selection is based on indications given both by the box plot and the descriptive analysis. The exposed group which is likely to differ to a large extent compared to the sham-exposed - based on mean difference - will be firstly subject to the Student t-test. When there is no statistical difference, no more comparisons are necessary. In the other case, maximally three comparisons have to be made independently from each other. This procedure reduces the number of executed comparisons.

Apart from the Student t-test, in order to correct totally for *multiple comparisons*, we performed the Dunnett test as a post-hoc. A multiple comparison test is a procedure for detailed examination of the differences between a set of means, usually after a general hypothesis that they are all equal, has been rejected. In our case, this hypothesis is evaluated by the ANOVA. 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 [4]. It is specifically designed for situations where all groups are to be pitted against one reference group *i.e.* the sham-exposed group in our experimental setting. 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 (*cfr.* Levene's test), the classic Dunnett test is used. On the contrary, when the variances are not assumed as equal, we run the Dunnett T3 test. This test may be applied when the number of subjects in each population is inferior to 50, which is our case.

Before, we have evaluated the conditions of validity:

- normality of the distribution: since the number of observations is in exceed of 20, the normal character of the distribution is assumed [5]. On the opposite, we performed a one-sample Kolmogorov-Smirnov test [6].
- as explained here above: the equality of the variances by means of a homogeneity test for variances (Levene's test).

Equal variances across samples is called homogeneity of variance. A robust statistical test is the Levene test [6]. This test is not dependent on the assumption of normality. As we have chosen a statistical significance level of 0.05, the null hypothesis H_0, stating that the variances are equal, must be accepted when $p > 0.05$. In the opposite, when $p < 0.05$, the variances have to be considered as unequal for the considered parameter in all groups.

We have focused on the statistical analysis of the following blood parameters: leucocytes or white blood cells (WBC), erythrocytes or red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), lymphocytes (LYM), neutrophils (NEUT), eosinophils (EOS), monocytes (MOC), reticulocytes (RETIC) and the stress hormones adrenocorticotropic hormone (ACTH) and corticosterone (CORTICO).

The values of the laboratory results concerning each selected parameter were subject to statistical analysis using Statistical Packages for Social Sciences (SPSS), version 15.0.

For each dependent variable we proceed like described in this section.

In order to make a distinction between the significance obtained by Student's t-test or Dunnett's test, the first is double asterisked in the synoptic tables in the next two sections.

1.2. Analysis of Period 1 (P1)

Blood samplings have been periodically performed during the on-going of the experiment. Fifteen parameters have been considered for haematological analysis as explained in Section 3.8 (Part I). All of them have been subject to statistical tests as mentioned in the former section.

After three months of exposure – this period is termed as P1 – two hours a day, no statistical significances ($p < 0.05$) were found for the following parameters: MCH, haematocrit, neutrophils, basophils, lymphocytes, reticulocytes, and corticosterone. This does not necessarily mean that the exposure has no effect on these parameters. Indeed, failing to find evidence against the null hypothesis H_0 means only that the data are consistent

with $H_{0,}$ not that we have clear evidence that H_0 is true. A significance level of 0.05 means simply "not likely to happen just by chance in 95% of the cases". Thus, a statistically significant difference implies there is statistical evidence that there is a difference. On the contrary, not finding any statistical evidence there is a difference between a parameter in an exposed group and the sham-exposed group does not necessarily constitute evidence there is no difference at all.

Blood parameters showing a statistically significant effect between exposed groups and sham-exposed group are taken into account for further discussion in the following sections.

In the course of the exposure campaign, blood sampling sessions have been carried out as planned. In case other parameters than those already mentioned in P1 show statistically significant differences, they are included in the discussion as well.

1.2.1. Monocytes P1

A more in-depth view by means of a box plot repress entation of the monocyte count and a graph regarding the mean values and standard deviations related to the first 3-month period of exposure is shown in Figure 1.1.

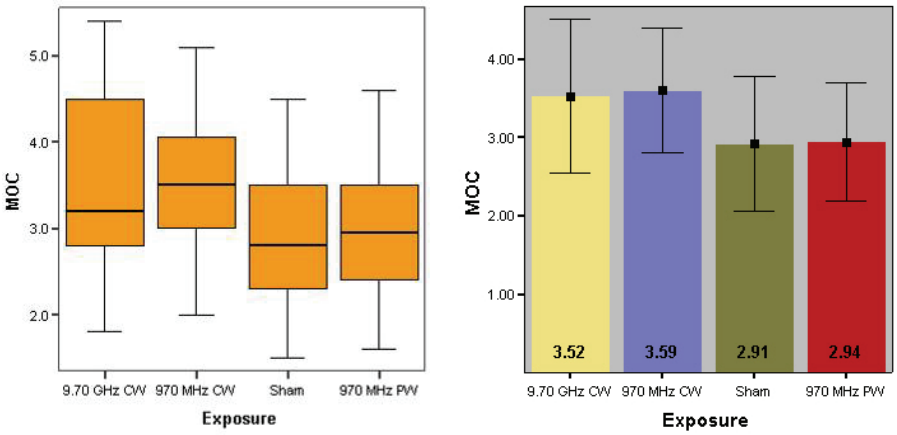

Figure 1.1: Descriptive analysis of monocyte count for different exposure types after 3-month exposure.

As can be seen in Figure 1.1, there is a main difference between the 970-MHz CW exposed group ($M = 3.594$, $SD = 0.7989$) and the sham-exposed group: the monocyte count in the peripheral blood of the 970-MHz CW exposed group has increased with 23.5 % during the first three months of exposure comparing to the sham-exposed rats.

Not only the 970-MHz CW group, but all the exposed groups show an increase in the monocyte level when compared to the sham-exposed group.

When comparing the mean value for the monocyte count of the 9.70-GHz CW group ($M = 3.523$, $SD = 0.9772$) with this of the sham-exposed group ($M = 2.910$, $SD = 0.8596$), there is a 21.1% increase in monocytes.

Comparing the monocytes values of the 970-MHz PW group ($M = 2.940$, $SD = 0.7546$) and the sham-exposed group learns that the increase in the exposed group is only 1.0%.

These findings have to be tested on their statistical significance, for the same we have chosen a Dunnett test.

Before enabling to run the Dunnett, the equality of variances in all our populations of rats has to be checked. This is done by means of Levene's test. With $p = 0.429$, the Levene test is not significant at the 0.05 level, indicating that the sample variances in all populations are equal. Indeed, the null hypothesis H_0, stating that there is no difference in variances related to the monocytes in all groups, can not be rejected; this implies that the variances are equal in all populations of rats and therefore one can proceed to the actual statistical test.

A second condition that has to be fulfilled before performing the Dunnett test, namely the normality of the distribution, has been verified and confirmed by a Kolmogorov-Smirnov test.

The mean difference between the sham-exposed group and the 970-MHz CW group, which represents a 23.5% increase in monocytes compared to the sham-exposed group, was found to be statistically significant ($p = 0.006$).

The 21.1% increase in monocytes in the 9.70-GHz CW group compared to the sham-exposed group is found to be statistically significant ($p = 0.017$).

The difference between the sham-exposed group and the 970-MHz PW group is not statistically significant ($p = 0.998$).

1.2.2. Haemoglobin (HGB) P1

As we found a statistically significant effect after the first three months of exposure regarding the monocytes, we decided to perform the same way for the other selected dependant variables. The haemoglobin concentration, expressed in g/100ml, is retained for further analysis. The box plot representation related to the first 3 months of exposure shows the descriptive statistics related to the haemoglobin concentration. This is depicted in

Figure 1.2. The descriptive statistical analysis shows that the blood haemoglobin concentration has risen with almost equal amplitude in the three exposed groups when compared to the sham-exposed group.

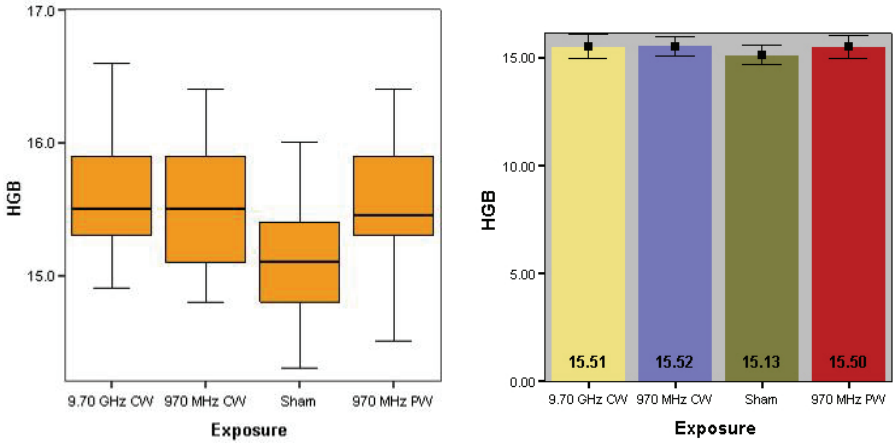

Figure 1.2: Descriptive analysis of haemoglobin concentration in the four groups after 3-month exposure.

The differences between the exposed groups are small, but the highest increase can be noticed in the 970-MHz CW group.

The difference between the mean value for haemoglobin concentration (mg/100ml) of 970-MHz CW exposed rats (M = 15.52, SD = 0.47) and that of the sham-exposed rats (M = 15.13, SD = 0.47) equals 0.39. This corresponds to a 2.6% higher haemoglobin concentration compared to the the sham-exposed group.

We found a mean difference of 0.38 between the haemoglobin concentration (mg/100ml) of the sham-exposed group (M = 15.13, SD = 0.47) and the 9.70-GHz CW group (M = 15.51, SD = 0.56). The 9.70-GHz CW group shows a 2.6% higher haemoglobin concentration.

Comparing the mean value for haemoglobin concentration of 970-MHz PW exposed rats (M = 15.50, SD = 0.54) with that of the sham-exposed rats (M = 15.13, SD = 0.47), points to a difference of 0.37. The 970-MHz PW group shows a 2.5% higher haemoglobin concentration compared to the sham-exposed group after a 3-month exposure period.

These findings have to be tested on statistical significance using a Dunnett test with a level of significance of 0.05.

The Levene test for equality of variances is statistically not significant (p = 0.943). This result indicates that the sample variances of all considered groups are equal.

Before, the normality of the distribution has been verified and confirmed.

The mean difference between the sham-exposed group and the three other exposed groups is statistically significant with a p-value of 0.010, 0.011, 0.014 for the 970-MHz CW group, the 9.70-GHz CW group, and the 970-MHz PW group, respectively.

1.2.3. Mean Corpuscular Haemoglobin Concentration P1

Figure 1.3 shows the Mean Corpuscular Haemoglobin Concentration (MCHC), expressed in g/dl, in the four groups of rats after the first period of three months of continuously exposure. One can observe a higher level of this variable in all exposed groups in comparison with the sham-exposed. The increase is in the order of 2%. This finding has to be tested on statistical significance using a Dunnett test with a level of significance of 0.05.

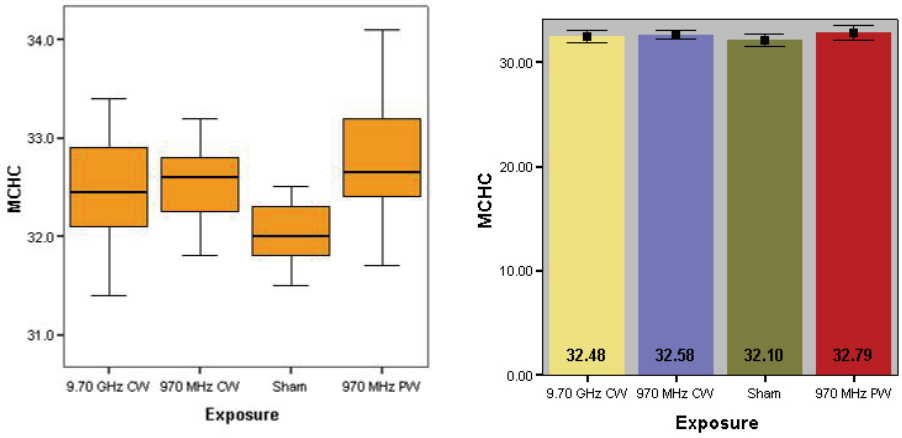

Figure 1.3: Descriptive analysis of mean corpuscular haemoglobin concentration in the four groups of rats after 3-month exposure.

The increase is the most pronounced in the 970-MHz PW group. We compared the mean value for the MCHC of 970-MHz PW exposed rats (M = 32.79, SD = 0.66) with this of the sham-exposed rats (M = 32.10, SD = 0.56). The mean difference represents a 2.2% increase in MCHC for the 970-MHz PW exposed group.

Secondly, the increased level of MCHC in the 970-MHz CW group is compared to the MCHC level in the sham-exposed group. The level of MCHC in the 970-MHz CW is 1.5 % higher.

Comparing the 9.70-GHz CW and the sham-exposed group shows a mean difference of 0.377, which represents a 1.2% increase compared to the sham-exposed group.

In order to see if these differences are statistically significant, a Dunnett test is used.

According to the Levene test for equality of variances, the sample variances are equal (p = 0.173).

Before, the normality of the distribution has been verified and confirmed.

The mean difference between the sham-exposed group and the three other exposed groups is statistically significant with a p-value of 0.004, 0.033, $2.41\ 10^{-5}$ for the 970-MHz CW group, the 9.70-GHz CW group, and the 970-MHz PW group, respectively.

1.2.4. Mean Corpuscular Volume (MCV) P1

Since we observed in the previous analysis statistically significant differences in haemoglobin related variables between exposed groups and the sham-exposed, it should be logical to put the mean corpuscular (cell) volume to the test. MCV is a measure for the mean volume per erythrocyte, by dividing the haematocrit value through the number of erythrocytes; it is expressed in femtoliter (fl, 10^{-15}l). As the haematocrit level of the sham-exposed group is high while the number of erythrocytes in the same group is rather at the lower level, it is not surprising that the MCV is high in the non-exposed group. This can be seen in Figure 1.4.

According to the Levene test for equality of variances, the sample variances are equal ($p = 0.583$).

Only the rats exposed to 970 MHz CW ($M = 52.87$, $SD = 1.36$) differ significantly from the MCV value in the sham-exposed rats ($M = 53.78$, $SD = 1.66$) according a Dunnett test ($p = 0.047$). The mean difference in MCV between the sham-exposed and the 970-MHz CW is 0.909, which is equivalent with a 1.7% decrease compared to the sham-exposed group.

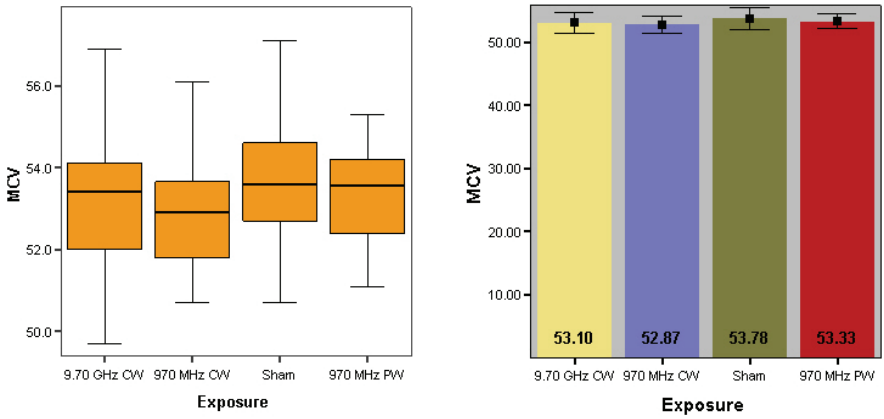

Figure 1.4: Mean corpuscular volume (fl) after 3 months of exposure in the 4 groups of rats.

1.2.5. Erythrocytes (RBC) P1

The results of the red blood cell count after a period of three months continuously exposure (two hours a day), are graphically represented in Figure 1.5. The figures have to be multiplied by 10^6 and stand for the number of erythrocytes per µl blood. All the exposed groups have a higher level of RBC than the sham-exposed group. The 970-MHz CW group (M = 9.02, SD = 0.39) differs to a larger extent in relation to the sham-exposed group (M = 8.75, SD = 0.41), followed by the 9.70-GHz CW group (M = 9.00, SD = 0.43).

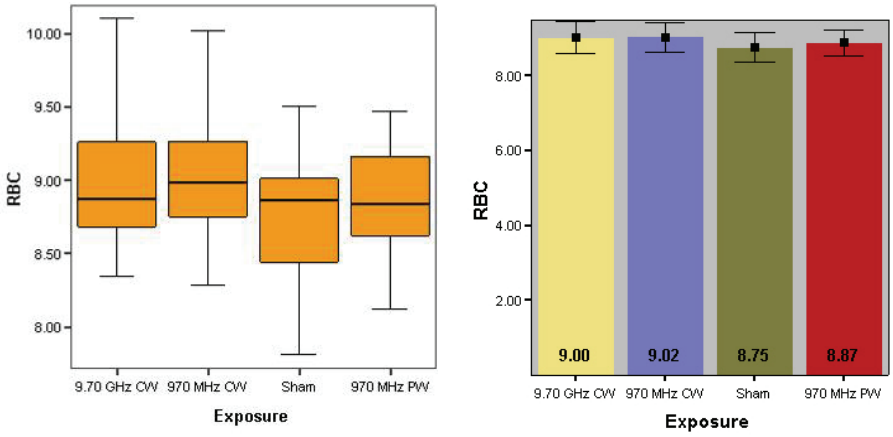

Figure 1.5: Red blood cell count after 3 months of exposure in the 4 groups of rats.

The variances in both samples are equal which is explained by Levene's test ($p = 0.741$). Since $p > 0.05$, the null hypothesis, stating that the variances in both groups are equal, can not be rejected. The number of rats in each group is sufficiently high to assume on a correct basis that the variable follows a normal distribution in both populations. Besides, the normality has been tested based on a Kolmogorov-Smirnov test.

The mean difference of 0.39 between the sham-exposed group and the 970-MHz CW group, which represents a 3.0% increase regarding to the 970-MHz CW group, was found to be statistically significant ($p = 0.025$).

The median of the 9.70-GHz CW group, almost equal to this of the former analysed group, showing a higher (2.9%) red blood cell count than the rats of the sham-exposed group is also statistically significant ($p = 0.037$).

The RBC in the 970-MHz PW group ($M = 8.8727$, $SD = 0.3448$) was 1.41% higher than in the sham-exposed group; this was found to be not statistically different from the sham-exposed group ($p = 0.475$).

1.2.6. Leucocytes (WBC) P1

The leucocyte count is expressed in 10^3 cells per µl. Figure 1.6 shows the white blood cell count after the first period of a three month continuous exposure at a rate of two hours per day.

The white blood cell count is higher in the two "continuous wave" groups compared to the sham-exposed group (M = 6.364, SD = 1.1438), *i.e.* in the 970-MHz CW (M = 7.059, SD = 1.4473) and the 9.70-GHz CW group (M = 6.843, SD = 1.0680).

Figure 1.6 shows that the WBC count in the peripheral blood of the 970-MHz CW exposed rats show a 10.9% higher white blood cell count during the first three months of exposure than the rats of the sham-exposed group.

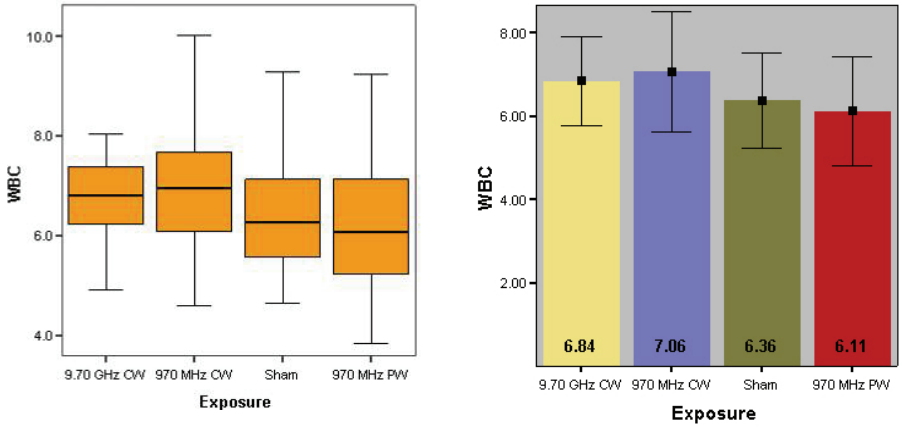

Figure 1.6: White blood cell count related to the four groups of rats, after 3 months of exposure.

After a 3-month exposure period, the 9.70-GHz CW group has increased with about 7.5 % comparing to the sham-exposed group.

The 970-MHz PW group (M = 6.108, SD = 1.3186) has a mean value of WBC which is 4% lower than the mean value for this parameter in the sham-exposed group.

These findings have to be tested on statistical significance using a Dunnett test with a 0.05 level of significance.

The Levene test for equality of variances does not point towards a statistically significant result (p = 0.239). This indicates that the sample variances are equal, because with p > 0.05, the null hypothesis, stating that there is no difference between the variances in white blood cell count between the 970-MHz CW exposed group and the sham-exposed group, can not be rejected.

Before, the normality of the distribution has been verified and confirmed.

The differences between the sham-exposed groups and the three other groups are not statistically significant, despite the 10.9% increase in WBC count in the 970-MHz CW group. The p-values are: p = 0.084, p = 0.321, p = 0.769 for the 970-MHz CW, the 9.70-GHz CW, and the 970-MHz PW exposure, respectively.

1.2.7. Eosinophils (EOS) P1

There is a main difference between the sham-exposed group and the real exposed groups in a sense that the eosinophils value is the most elevated for the non-exposed rats (Figure 1.7). The eosinophil count in the peripheral blood of the 970-MHz CW is 16.8 % lower than in the sham-exposed group after the first three months of exposure; the 9.70-GHz CW group shows a decrease of 11.9% compared to the sham-exposed rats and the 970-MHz PW has a 7.4% decrease compared to the sham-exposed group.

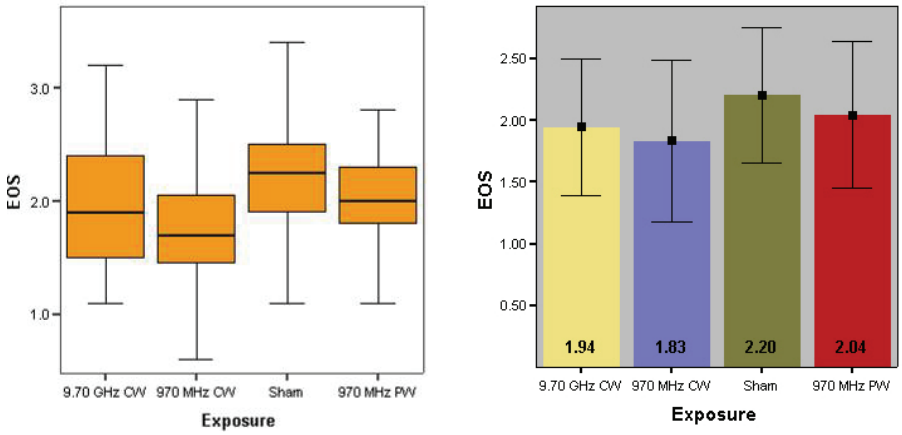

Figure 1.7: Eosinophil count for different exposure types after 3-month exposure.

These findings have to be tested on their statistical significance by means of a Dunnett test.

The variances of all groups can be considered as equal according to the p-value resulting from the Levene test ($p = 0.856$). Therefore, one can not reject the null hypothesis which states that the variances in both populations are equal.

The normality of the distribution has been verified and confirmed before performing the Dunnett test.

The mean difference in eosinophil count of 0.37, which represents a decrease of 16.9%, between the sham-exposed group ($M = 2.20$, $SD = 0.55$) and the 970-MHz CW group ($M = 1.83$, $SD = 0.65$) was found to be statistically significant ($p = 0.042$).

The 11.9% decrease in eosinophil count in the 9.70-GHz CW ($M = 1.94$, $SD = 0.56$) group in comparison with the sham-exposed group was not statistically significant ($p = 0.217$).

The 7.4% decrease in the 970-MHz PW ($M = 2.04$, $SD = 0.60$) compared to the sham-exposed group was not statistically significant either ($p = 0.577$).

1.2.8. ACTH P1

It is still an open question whether the overall stress level is affected by low-level electromagnetic fields. Results of animal experiments dealing with this issue are still being debated. Overall, as already mentioned in section 3.8.2 of part I, the available evidence is suggestive of subtle effects, but is not conclusive, and some inconsistencies exist regarding the direction of change of the reported effects.

The descriptive statistical analysis graphically depicted in Figure 1.8, displays the ACTH (pg/ml) in the peripheral blood of the four groups of rats after the first three month of exposure. A decreased expression is detected in all the exposed groups compared to the sham-exposed group. The highest difference in ACTH value is found between the sham-exposed group (M = 216.61, SD = 123.90) and the 970-MHz PW (M = 149.30, SD = 54.57) exposed group, where the level is 31.1% decreased.

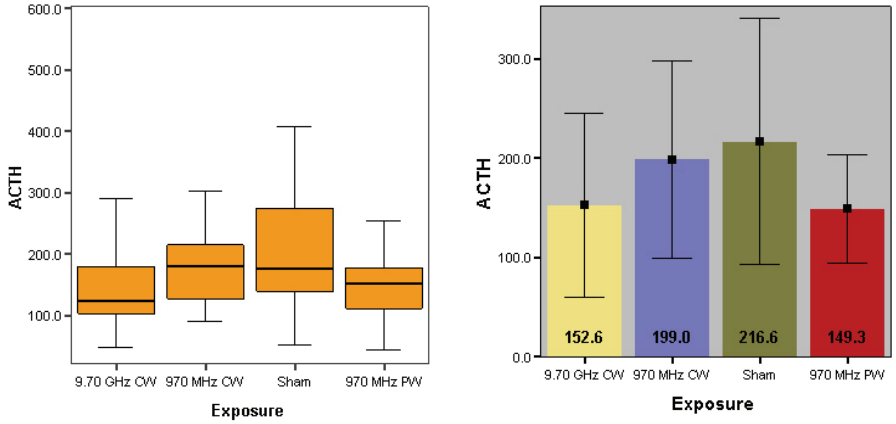

Figure 1.8: Descriptive analysis of ACTH (pg/ml) in the four groups after 3-month exposure.

The difference between the 9.70-GHz CW group and the sham-exposed group is 29.5%, whereas the decrease in the 970-MHz CW group is about 8.1%.

It is worthwhile to investigate these findings on their statistical significance, using a Dunnett test. Statistical significance was defined as $p < 0.05$.

The variances of both populations have to be considered as not equal according to the Levene test ($p = 0.034$). As a result we have to use the Dunnett T3 test for unequal variances as post-hoc test.

Before performing the Dunnett T3 test, the normality of the distribution has been verified and confirmed.

The mean difference of 67.31 between the sham-exposed group and the 970-MHz PW group, was found to be nearly significant ($p = 0.055$) for the parameter of the ACTH concentration, after the first three months of exposure.

Furthermore, the decreased expression of ACTH (29.5%) in the peripheral blood of the 9.70-GHz CW exposed rats ($M = 152.62$, $SD = 92.44$) in comparison with the sham-exposed group was found to be statistically insignificant ($p = 0.151$).

The 8.1% decrease in ACTH concerning the 970-MHz CW exposed rats ($M = 198.99$, $SD = 99.43$) was not proven to be statistically significant in comparison with the sham-exposed group ($p = 0.990$).

1.2.9. Synopsis

Until now, we have analysed a selected number of parameters after the first three months of exposure. Most of them are related to leucocytes, erythrocytes and stress hormones. We have compared the values of the exposed groups with those of the sham-exposed group and have observed statistical significances between both groups for some of the parameters. We found statistically significant differences for the following parameters: monocytes, leucocytes, erythrocytes, haemoglobin and its derivatives MCV,

MCHC, and eosinophils. A synoptic table (Table 1.1) summarizes those parameters which have demonstrated statistical differences (*, **) in comparison with the sham-exposed group.

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 10.9%**	- 4.0%	+ 7.5%
Monocytes	+ 23.5%*	+ 1.0%	+ 21.1%*
Erythrocytes (RBC)	+ 3.0%*	+ 1.4%	+ 2.9%*
Haemoglobin	+ 2.6%*	+ 2.5%*	+ 2.6%*
MCV	- 1.7%*	- 0.8%	- 1.3%
MCHC	+ 1.5%*	+ 2.2%*	+ 1.2%*
ACTH	- 8.1%	- 31.1%**	- 29.5%**
Eosinophils	- 16.8%*	- 7.4%	- 11.9%

Table 1.1: Parameters presenting a statistically significant difference according to Dunnett (*) or Student (* & **) compared to the sham-exposed group, after 3 months of exposure.

In the next series we inquire into the continuance of the observed tendencies after a prolonged exposure period of 8 months in total. The format of the reporting of the investigations is analogous to that in the previous considered period of three months.

The statistical analysis related to periods P2-P5 can be consulted in the appendix at the end of this work. A synopsis of these findings is presented in the following section. These results are further discussed in Chapter 2.

1.3. Analysis of Period P2 till P5: Results

In the present section we summarize the results of the analysis of the different haematological parameters from exposure period P2 till exposure period P5. In favour of the readability of the text, we have chosen to present these outcomes here only synoptically. The performed statistical procedure is analogous to this described in the former section related to the first exposure period (P1), *i.e.* after three months of exposure (two hours a day, seven days a week). More details about the results of the statistical analysis can be found in the annexes.

Similar to the first period, the results of the analysis are submitted to an ANOVA test for multiple comparisons. A Dunnett test is used as post-hoc to correct for multiple comparisons. Statistically significant results according to Dunnett's test are marked with *, according to Student's t-test with * and **.

1.3.1. Synopsis P2

After 8 months of exposure - this period is called exposure period P2 - a second blood sampling is performed.

The ANOVA test shows a significantly difference between the means of the following parameters: monocytes, erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC).

Similar to the first three-month period of exposure, an increased expression of monocytes is detected in all the three real exposed groups in comparison with the sham-exposed group. This can be seen in Table 1.2.

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 2.6%	- 7.0%	- 1.2%
Monocytes	+ 20.6%*	+ 8.4%	+ 17.1%**
Erythrocytes (RBC)	- 1.8%	- 4.0%**	+ 0.4%
Haemoglobin	- 1.7%	- 2.9%	+ 0.6%
MCV	- 1.5%**	+ 0.4%	- 2.5%*
MCHC	+ 1.9%*	+ 1.0%**	+ 3.6%*
ACTH	- 11.2%	- 12.0%	- 11.3%
Reticulocytes	+ 6.18%	+ 14,01%*	+ 5.31%
Eosinophils	- 4.6%	- 12.6%	- 8.2%

Table 1.2: Parameters presenting a statistically significant difference according to Dunnett (*) or Student (* & **), compared to the sham-exposed group, after 8 months of exposure.

The most obvious change attributable to the microwave exposure is the sharp increase (20.6%) in the monocyte count of the 970-MHz CW group compared to the sham-exposed group. Another meaningful increase is found in the 9.70-GHz CW group where the monocyte count is 17.1% higher than in the sham-exposed group. The increase in the 970-MHz PW group is about 8.4%. Further, we can observe a decrease of the order of 10% in ACTH; although this figure is not statistically significant, it is still worthwhile to mention.

Another remarkable fact is the sharp increase in reticulocyte count in all exposed groups compared to the sham-exposed group. The 14% increase in the 970-MHz PW group is statistically significant. Furthermore, there are slight, but significant increases in erythrocyte related parameters.

In the current of the sixth month of the exposure campaign, the amplifier connected to the 9.70-GHz microwave generator fell down. Despite of frenetic attempts, the apparatus could not be repaired anymore. However, we carried out the protocol as it was planned: every day, the 9.70-GHz CW group continued to be placed into the exposure unit during two hours and blood samplings were performed as foreseen.

1.3.2. Synopsis P3

After 11 months of exposure, we still observe an increase in monocytes in all the exposed groups compared to the sham-exposed group (Table 1.3).

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 9.9%	- 0.9%	+ 3.6%
Monocytes	+ 13.0%	+ 8.8%	+ 9.6%
Erythrocytes (RBC)	- 1.3%	- 0.1%	- 0.6%
Haemoglobin	- 1.3%	0.0%	- 0.6%
Haematocrit	- 3.4%*	- 1.6%	- 2.6%*
MCV	- 1.8%*	- 1.1%	- 1.4%
MCHC	+ 2.7%*	+ 2.0%*	+ 2.3%*
Retic#	- 2.5%	- 0.8%	- 12.9%*
ACTH	+ 6.9%	+ 28.4%**	+ 7.1%
Corticosterone	- 2.1%	+ 20.6 %**	- 6.2%
Eosinophils	- 11.9%	- 11.5%	- 8.0%

Table 1.3: Parameters presenting a statistically significant difference according to Dunnett (*) or Student (* & **), compared to the sham-exposed group, after 11 months of exposure.

This is a similar tendency as in the former exposure periods. The rise in the 970-MHz CW group is the most obvious (13%), followed by the 9.70-GHz CW group (9.6%). The increase concerning the 970-MHz PW group is 8.8%.

In addition to erythrocytes-derived indices, statistically significant differences can be observed in the exposed groups when comparing to the sham-exposed. In contrast to the first exposed period of three months, a statistically significant increase related to both corticosterone and ACTH is noticed in the pulse-amplitude modulated exposed group.

1.3.3. Synopsis P4

Like in the former exposure period of 11 months, only a marginally increase (nearly 2 %) related to the blood haemoglobin concentration is observed between exposed groups and sham-exposed group. We still observe an increase in monocytes (9 – 16.4%) in all exposed groups compared to the sham-exposed group.

The Dunnett shows significant differences ($p < 0.05$) in the means of the following parameters: leucocytes, haematocrit, reticulocytes and nearly the erythrocytes ($p = 0.052$). This can be seen in Table 1.4.

Though, a result which only slightly gets over the artificially chosen significance level of 0.05 can not be neglected just like that. A p-value equal to 0.052 means that the result could be only due to chance in 5.2% of the cases. This still implicates that the result is not attributable to chance in 94.8% of the cases.

A substantial decrease in eosinophils continues to show up, although this effect is not statistically significant.

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 14.1%*	+ 4.0%	+ 17.2%*
Monocytes	+ 9.0%	+ 14.1%**	+ 16.6%**
Erythrocytes (RBC)	+ 2.7%**	+ 0.3%	+ 2.6%**
Haemoglobin	+ 1.9%	+ 1.9%	+ 2.4%**
Haematocrit	+ 2.7%*	+ 1.4%	+ 2.1%
MCV	- 0.02%	+ 1.0%	- 0.5%
MCHC	- 0.8%	+ 0.5%	+ 0.3%
Retic#	+ 11.0%*	+ 5.0%	+ 6.3%
ACTH	+ 1.1%	+ 19.2%	+ 3.8%
Cortico	- 1.8%	+ 7.5%	- 4.3%
Eosinophils	- 15.6%	- 14.8%	- 11.1%

Table 1.4: Parameters presenting a statistically significant difference according to Dunnett (*) or Student (* & **), compared to the sham-exposed group, after 14 months of exposure.

1.3.4. Synopsis P5

After 18 months of exposure, both the 970-MHz CW and 970-MHz PW groups present an increase in leucocyte count of 33% and 21%, respectively (Table 1.5.). This difference related to the sham-exposed group is statistically significant. The reticulocyte count in the 970-MHz PW group is significantly increased with about 15% compared to the sham-exposed group (only according to Student). Blood sampling analysis further show an increase of about 30% in neutrophils in all exposed groups. For the first time during this long-term exposure, lymphocytes show a statistically significant

decrease of about 15%. Details on other analysed parameters can be seen in Table 1.5.

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 33.6%*	+ 21.0%**	+ 7.7%
Monocytes	+ 12.5%	+ 3.3%	+ 2.1%
Erythrocytes (RBC)	- 1.4%	- 1.1%	+ 3.6%
Haemoglobin	- 3.6%	- 1.6%	+ 3.1%
Haematocrit	- 3.3%	- 0.7%	+ 0.8%
MCV	- 1.9%	+ 0.6%	- 1.2%
MCHC	- 0.7%	- 1.3%*	+ 1.9%*
Retic#	+ 1.3%	+ 15.5%**	+ 1.3%
Neutrophils	+ 27.9*	+ 26.4%*	+ 34.7*
Lymphocytes	- 13.0%*	- 11.6%*	- 15.1%*
ACTH	+ 23.2%	- 6.9%	+ 1.3%
Cortico	- 17.4%	- 8.8%	- 3.3%
Eosinophils	+ 19.4%	+ 8.1%	+ 2.5%

Table 1.5: Parameters presenting a statistically significant difference according to Dunnett (*) or Student (* & **), compared to the sham-exposed group, after 18 months of exposure.

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Chapter 2 Discussion of the Statistically Significant Results

In what follows, we shall discuss parameter per parameter along the whole exposure period. *i.e.* from period P1 to period P5.

Although we realize that absence of a statistically significant result does not necessarily mean that there is no effect at all, we retain for discussion those physiological parameters which are significantly different compared to the sham-exposed group from the first exposure period on: monocytes, erythrocytes, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin concentration, eosinophil count and ACTH.

2.1. Monocytes

In the first period (P1), *i.e.* after three months of exposure, the increase in monocytes is the most obvious result. We noticed a 23.5% increase and a 21% increase in the 970-MHz CW group and the 9.70-GHz CW group, respectively. Both results are statistically significant. The monocyte count in the 970-MHz PW group has only increased by 1% compared to the sham-exposed group. Both CW exposure groups react in the same way: an increase of about 20% in monocyte count.

This pattern can be observed again after the following period of 5 months of exposure (P2). In the 970-MHz CW group, the monocyte count is increased by 20.6% compared to the sham-exposed group which is statistically significant. The monocyte count in the 9.70-GHz group shows a 17.1% increase, whereas the 970-MHz PW exhibits an 8.4% increase.

After 11 months of exposure (P3), no more significant differences between exposed groups and sham-exposed group could be detected.

After 14 months of exposure (P4), the 970-MHz PW group shows a 14.7% increase in monocyte count. The 9.70-GHz CW group exhibits a 16.6% increase.

After a total exposure period of 18 months (P5) there is no statistically significant difference between each of the exposed groups and the sham-exposed group left.

These observations are shown in Figure 2.1. and summarized in Table 2.1.

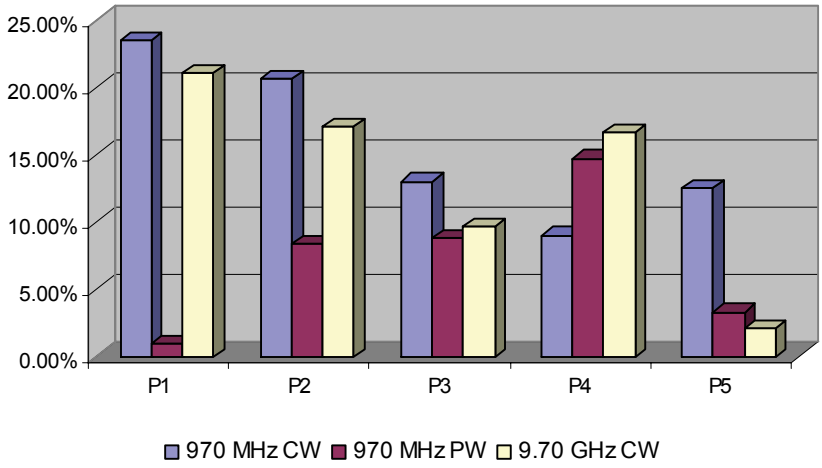

Figure 2.1: Alterations in monocyte count in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

Exposure period	970 MHz CW	970 MHz PW	9.70 GHz CW
Monocytes P1	+ 23.5%*	+ 1.0%	+ 21.1%*
Monocytes P2	+ 20.6%*	+ 8.4%	+ 17.1%**
Monocytes P3	+ 13.0%	+ 8.8%	+ 9.6%
Monocytes P4	+ 9.0%	+ 14.7 %**	+ 16.6%**
Monocytes P5	+ 12.5%	+ 3.3%	+ 2.1%

Table 2.1: Alterations in monocyte count in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Dunnett (*) or Student (* & **), at $p < 0.05$.

It is remarkable that the monocyte count shows an increase in each of the exposed groups compared to the sham-exposed group. The earliest effect is observed in both CW groups. The effect with the largest amplitude is noticed in both CW groups too. The reaction in the PW group seems to be delayed in time. The first rupture in a series of statistically significant effects takes place after 11 month of exposure (P3): the monocyte count is still higher than in the sham-exposed group, but without any significant statistical difference.

Monocytes play an important role in defending the body against foreign organisms and form one of the key players of the immune system.

Monocytes are found in all tissues (macrophages) and also circulate in the blood. They are generally the first cells to encounter a foreign substance in the body. They non-specifically engulf such materials, as well as scavenge normal cellular debris, and degrade them, using powerful hydrolytic enzymes and oxidative attack.

In the scientific literature, less is found on the effects of microwave exposure on monocytes.

Switzer and Mitchell [1] exposed female rats to 2.45 GHz CW in a cavity (resultant SAR 2.3 W/kg). The rats were exposed simultaneously, but restrained individually in polystyrene cylinders. Animals were exposed 5 hours per day for 110 days. They found higher monocytes counts in all exposed rats.

In another study, Barron and co-workers performed a 13 years' follow-up study among radar personnel employed by an aircraft company. Radar personnel were grouped by years of exposure and compared to controls of similar age. A marked increase in monocytes (above 6%) was detected in exposed persons [2].

The other results of our study are summarized and briefly discussed in what follows.

2.2. Erythrocytes (RBC)

The erythrocyte count is increased after the first exposure period of three months in all the exposed groups compared to the sham-exposed group. Statistically significant increases are found in both the CW groups. This can be observed in Figure 2.2 and Table 2.2.

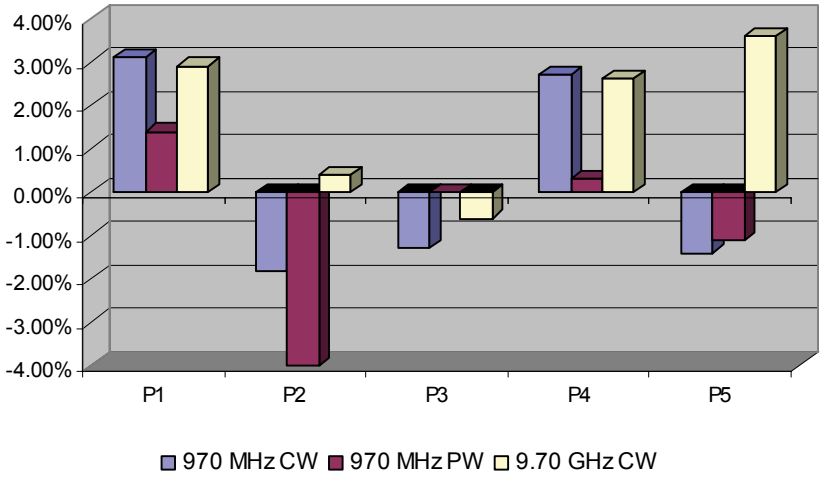

Figure 2.2: Alterations in erythrocyte (RBC) count in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

Exposure period	970 MHz CW	970 MHz PW	9.70 GHz CW
Erythrocytes (RBC) P1	+ 3.1%*	+ 1.4%	+ 2.9%*
Erythrocytes (RBC) P2	- 1.8%	- 4%**	+ 0.4%
Erythrocytes (RBC) P3	- 1.3%	0%	- 0.6%
Erythrocytes (RBC) P4	+ 2.7%**	+ 0.3 %	+ 2.6%**
Erythrocytes (RBC) P5	- 1.4%	- 1.1%	+ 3.6%

Table 2.2: Alterations in erythrocyte (RBC) count in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Dunnett (*) or Student (* & **), at $p < 0.05$.

The increase of erythrocytes is an effect that can be found in previous scientific literature where rats have been exposed to 2.45 GHz CW for one month, two hours a day, at 100 W/m² [1, 3]. An increased RBC count has been found by Deichmann *et al.* who exposed rats for 11 consecutive days (10 minutes to 7.5 hours) to 24 GHz PW at 100 W/m² [4]. Busljeta *et al.* [5]

observed an increased erythrocyte count in peripheral blood of male *Wistar* rats after exposure to 2.45 GHz (2 hours, daily, 50-100 W/m², SAR 1-2 W/kg, 15 days).

Sedehi *et al.* [6] found a statistically significant increase in erythrocyte count in male rats that have been exposed to 2.45 GHz, thirteen minutes a day, during one year at a power density of 100 W/m². The increase in erythrocyte count in a simultaneously exposed similar group of male rats at 10 W/m² was not statistically significant.

Kitsovskaya and co-workers found no changes in rat haematological parameters after a seven-month exposure (one hour a day) to 3 GHz PW at 100 W/m². When the power density increased to 400 W/m² at a decreasing exposure period (20 days, 15 minutes per day) a decrease in RBC count was observed [7].

After 8 months of exposure, we found a statistically significant decrease in RBC count in the 970-MHz PW group.

No changes were found anymore by Djordjevic after a three-month exposure period of rats (one hour a day) to 2.4 GHz CW at 50 W/m² [8]. In contrast, Matausic *et al.* [9] reported an increased erythrocyte count in the peripheral blood of exposed rats (2.45 GHz, 100 W/m², 2 hours a day, 30 days).

As can be noticed from the references here above, conflicting reports on the effects of microwave exposure on the peripheral blood picture exist. The differences among the results obtained may be ascribed to species specificity and/or various exposure conditions. In general, it is difficult to compare the results we obtained with comparable scientific studies, just because of the lack of common elements. Frequency differs and, most often, the exposure level in the reference works is at least one order of magnitude higher, what makes it an obvious thermal exposure.

2.3. Leucocytes (WBC)

Besides the observed effect on monocytes, we found differences in leucocyte counts too from the first exposure period on. As can be seen in Figure 2.3 and Table 2.3, during the entire exposure period, leucocytes are increased compared to the sham-exposed group, in general in both of the CW exposures.

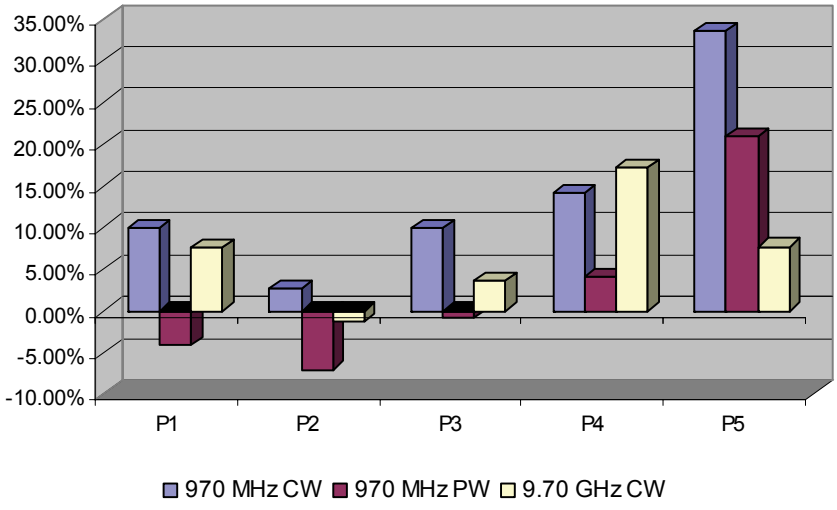

Figure 2.3: Alterations in leucocyte (WBC) count in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

Exposure Period	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC) P1	+ 9.9%**	- 4.0%	+ 7.5%
Leucocytes (WBC) P2	+ 2.6%	- 7.0%	- 1.2%
Leucocytes (WBC) P3	+ 10.0%	- 0.9%	+ 3.6%
Leucocytes (WBC) P4	+ 14.1%*	+ 4.0%	+ 17.2%*
Leucocytes (WBC) P5	+ 33.6%*	+ 21.0%**	+ 7.7%

Table 2.3: Alterations in leucocyte (WBC) count in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Dunnett (*) or Student (* & **), at $p < 0.05$.

After three months of exposure, the leucocyte count is nearly significantly increased in the 970-MHz CW group. It remains increased during the next two periods, but without statistical significance. After 14 and 18 months of exposure (P4 and P5) the leucocyte count turns to a statistically significant increase. The first statistically significant increase in the 9.70-GHz CW group can be observed after 14 months of exposure.

It is difficult to compare our results with those of other investigators because exposure set-ups and selected parameters are often unique. Besides, to our knowledge, there are not so many studies considering whole body exposure of rats. Matausic and co-workers [9] performed whole body exposure of male *Wistar* rats (2.45 GHz, 5 days a week, 2 hours a day, average power density 100 W/m²) during 30 days. Total leucocyte count was decreased ($p < 0.05$) in exposed animals, compared to the sham-exposed group.

Their conclusion is that microwave exposure may affect the haematological parameters of exposed animals. This can be partially subscribed referring to our results.

2.4. Mean corpuscular volume (MCV)

Another parameter which showed a statistically significance already after the first three months of exposure is the mean corpuscular volume (MCV), a measure for the average volume of an erythrocyte. It can be calculated by dividing the haematocrit value by the number of erythrocytes. It is expressed in femtoliter (fl). Apparently, the general tendency over the five considered exposure periods is a decrease of the MCV in the exposed groups compared to the sham-exposed group (Figure 2.4, Table 2.4).

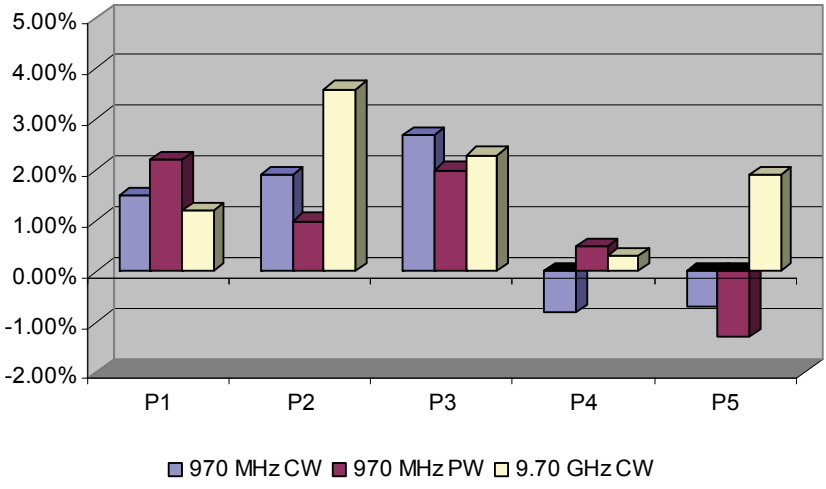

Figure 2.4: Alterations in MCV (fl) in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

Exposure period	970 MHz CW	970 MHz PW	9.70 GHz CW
MCV P1	- 1.7%*	- 0.8%	- 1.3%
MCV P2	- 1.5%**	+ 0.4%	- 2.5%*
MCV P3	- 1.8%*	- 1.1%	- 1.4%
MCV P4	- 0.02%	+ 1.0%	- 0.5%
MCV P5	- 1.9%	+ 0.6%	- 1.2%

Table 2.4: Alterations in MCV (fl) in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Dunnett (*) or Student (* & **), at $p < 0.05$.

Most of the statistically significant differences are seen in the 970-MHz CW group after 3 months and 11 months of exposure. No statistically significant results are found in the PW group.

2.5. Mean Corpuscular Haemoglobin Concentration (MCHC)

Considered over all periods, from P1 to P5, the MCHC parameter showed most often statistically different values compared to the sham-exposed group.

The MCHC measures the average concentration of haemoglobin in an erythrocyte. This index is obtained by dividing the haemoglobin by the haematocrit. MCHC is expressed in gram per decilitre (g/dl). Most values are increased when compared to the sham-exposed group as is shown in Figure 2.5 and Table 2.5.

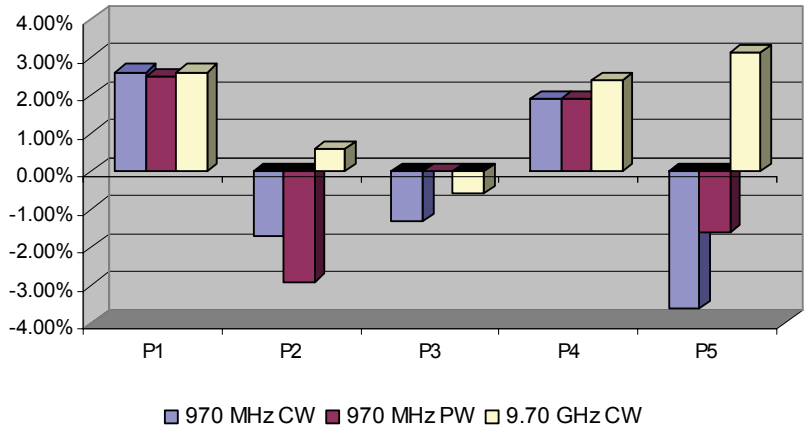

Figure 2.5: Alterations in MCHC (g/dl) in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

Exposure period	970 MHz CW	970 MHz PW	9.70 GHz CW
MCHC P1	+ 1.5%*	+ 2.2%*	+ 1.2%*
MCHC P2	+ 1.9%*	+ 1.0%**	+ 3.6%*
MCHC P3	+ 2.7%*	+ 2.0%*	+ 2.3%*
MCHC P4	- 0.8%	+ 0.5%	+ 0.3%
MCHC P5	- 0.7%	- 1.3%*	+ 1.9%*

Table 2.5: Alterations in MCHC (g/dl) in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Dunnett (*) or Student (* & **), at $p < 0.05$.

In each of the first three exposure periods, statistically significant differences are observed. Continuing the exposure for three months (P4) does not result in significant differences between exposed and sham-exposed group. After 18 months (P5) of exposure significant differences can be observed in the 970-MHz PW (1.3% decrease) and the 9.70-GHz CW group (1.9% increase).

2.6. Haemoglobin (HGB)

Haemoglobin concentration differences were significant in the first, fourth and fifth period.

After 3 months of exposure an almost identically increase in all of the three exposed groups can be noticed, compared to the sham-exposed group. These results are statistically significant. An overview of all exposed periods is displayed in Figure 2.6 and Table 2.6.

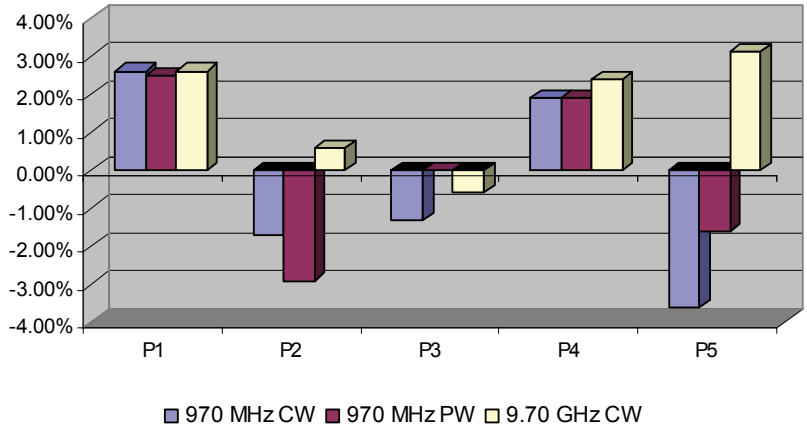

Figure 2.6: Alterations in haemoglobin concentration (g/dl) in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

Exposure period	970 MHz CW	970 MHz PW	9.70 GHz CW
HGB P1	+ 2.6%*	+ 2.5%*	+ 2.6%*
HGB P2	- 1.7%	- 2.9%	+ 0.6%
HGB P3	- 1.3%	- 0.0%	- 0.6%
HGB P4	+ 1.9%	+ 1.9%	+ 2.4%**
HGB P5	- 3.6%	- 1.6%	+ 3.1%

Table 2.6: Alterations in haemoglobin concentration (g/dl) in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Dunnett (*) or Student (* & **), at $p < 0.05$.

While we observed an increase in haemoglobin concentration after the first three months of exposure, the haemoglobin concentration showed a decrease in all groups after 11 months of exposure (P3). After 14 months of exposure (P4) the results turn to an increase in all exposed groups. After the first three months of exposure, however, no consistent pattern is detectable anymore. Busljeta and co-workers [5] found an increased haemoglobin concentration in peripheral blood of male *Wistar* albino rats after 8 and 15 days of exposure (2.45 GHz CW, 2 hours, daily, 50-100 W/m²). Sedehi *et al.* reported significantly increased haemoglobin concentration in male rats (2.45 GHz, 30 minutes per day, 1 year, 100 W/m²) [6].

2.7. Eosinophils (EOS)

What the eosinophils concerns, the only statistically significant difference between the 970-MHz CW group and the sham-exposed group appears after three months of exposure, where a decrease of 16.8% is observed (Figure 2.7, Table 2.7).

A decrease in eosinophils continues to be seen in most of the exposed groups, when compared to the sham-exposed group, but without any statistically significance.

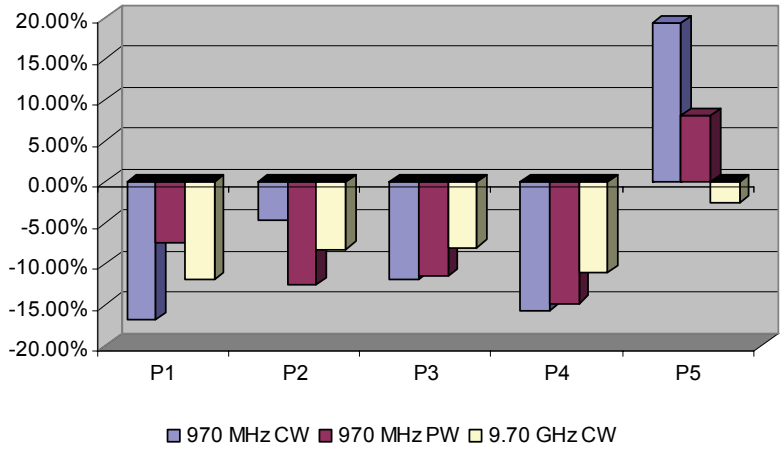

Figure 2.7: Alterations in eosinophil count in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

Exposure period	970 MHz CW	970 MHz PW	9.70 GHz CW
Eosinophils P1	- 16.8%*	- 7.4%	- 11.9%
Eosinophils P2	- 4.6%	- 12.6%	- 8.2%
Eosinophils P3	- 11.9%	- 11.5%	- 8.0%
Eosinophils P4	- 15.6%	- 14.8%	- 11.1%
Eosinophils P5	+ 19.4%	+ 8.1%	- 2.5%

Table 2.7: Alterations in eosinophil count in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Dunnett (*) and Student (*), at $p < 0.05$.

2.8. ACTH

The last parameter which differs nearly significantly between exposed and sham-exposed groups from the first period on is the ACTH concentration.

During the first period of exposure, *i.e.* after 3 months, there is a decrease in blood ACTH concentration of all exposed groups when compared to the sham-exposed group. The decrease in the 970-MHz PW group is almost statistically significant, with a p-value of 0.055. After 8 months of exposure (P2), the same tendency in the three groups can be observed, but without any statistically significance. After 11 months of exposure (P3), a rupture in the pattern becomes visible when an increased ACTH level in all exposed groups can be observed. This can be seen in Figure 2.8 and Table 2.8.

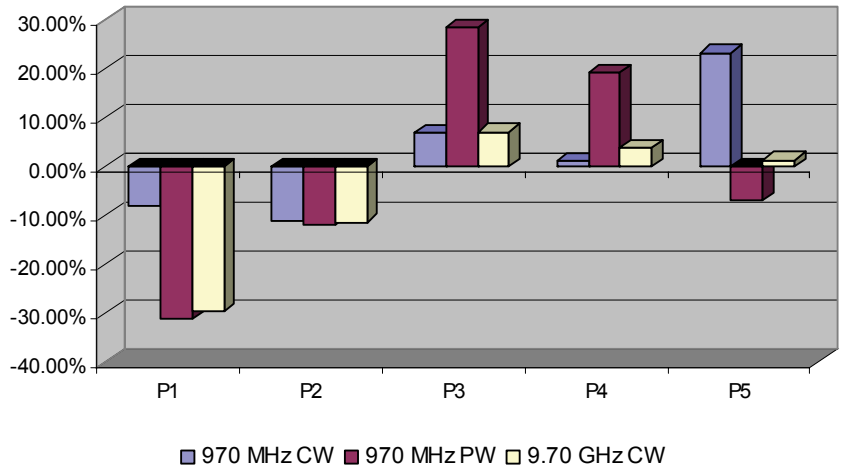

Figure 2.8 Alterations in ACTH in exposed groups compared to the sham-exposed group (0%) for the different considered exposure periods.

The increase in all exposed groups continues after 14 months of exposure and after 18 months, except for the PW group where a decrease of almost 7% is recorded.

Exposure period	970 MHz CW	970 MHz PW	9.70 GHz CW
ACTH P1	- 8.1%	- 31.1%**	- 29.5%**
ACTH P2	- 11.2%	- 12.0%	- 11.3%
ACTH P3	+ 6.9%	+ 28.4%**	+ 7.1%
ACTH P4	+ 1.1%	+ 19.2%	+ 3.8%
ACTH P5	+ 23.2%	- 6.9%	+ 1.3%

Table 2.8: Alterations in ACTH in exposed groups compared to the sham-exposed group for the different considered exposure periods, with indicating of statistically significant effects according to Student (**), at $p < 0.05$.

2.9. The Underlying Mechanism for Biological Effects: a Hypothesis

The underlying mechanisms for the changes in haematological parameters which we observed are not clear.

In the 80's most of the animal studies on the haematopoetic system found effects mainly when a significant rise in body temperature has been observed. Few effects have been found in the absence of a detectable increase in temperature. Most of the scientific articles deal with an increase in neutrophils and a decrease in lymphocytes after microwave exposure [10].

A suggested hypothesis, which has never been tested, is that whole body radiofrequency exposure induces heat stress that activates the hypothalamic-hypophysial adrenal axis to trigger the release of adrenal steroids in the blood, leading to transient alterations in blood cell counts and other haematopoietic changes associated with microwave exposure [10].

As explained in Section 3.5 of part I, we have chosen an exposure level at which no measurable temperature changes could be detected between the rats of the exposed group and the sham-exposed group. Besides, when

comparing our exposure level with the so-called low-thermal animal studies in the literature, it can be stated that we did the exposure under low-thermal conditions. Therefore, we do not put forward a thermal action mechanism as a possible explanation for our results.

In contrast to our study most of the others are rather short-termed, where, depending from case to case, the animals are only exposed from a few hours to a few months maximum, at a rate of one or two hours per day. So it is possible that due to the prolonged exposure period to which our rats have been submitted, a cumulative effect could have occurred. Due to the long-term character of the exposure it is possible that a slight, but persistent injury at cell level could not have been fully neutralized by the body's repair mechanism before another exposure takes place. It is the summation of such subtle effects – which would otherwise have been overlooked due to compensation and/or cell repairing mechanisms – that could have led to a detectable biological effect. This can be an explanation why we have reported some biological effects under low-thermal exposure conditions where others failed to show any.

Long-term microwave exposure might cause disturbance in erythrocyte maturation and proliferation. It could be hypothesized that the precursor cells of the leucocytes and erythrocytes are affected by microwave radiation, which could explain some of our findings of altered haematological parameters like monocytes and erythrocytes.

Based on the observations of an increased monocyte count in all exposed groups, there may be hypothesized that the chronic exposure to low-thermal microwaves may induce stimulation of the proliferative process in red bone marrow. Haematopoiesis takes place in bone marrow. As these tissues with blood forming capacity are situated periphere to the body, microwaves can easily penetrate them and possibly interfere with the genesis of blood cells.

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Part III Behaviour and Mortality

Chapter 1 Behavioural Study

1.1. Preliminary Results of a Trial Session – Onset for Further Research

A survey of existent literature on biological effects of microwaves learns that these are not only physiological. Many research reports deal with behavioural effects, including memory. The study performed by the Netherlands Organization for Applied Research (TNO) regarding the effects of GSM (Global System for Mobile communications) and UMTS (Universal Mobile Telecommunications System) signals on well-being and cognition in man aroused considerable controversy. The authors found a statistically significant relation between exposure to signals from an equivalent UMTS base station and decrease of measures of well-being. Furthermore, they found differences in diverse cognitive functions, for exposure to GSM signals and UMTS signals as well [1]. A Swiss study [2] found no effects of exposure to UMTS signals in healthy adults. However, this latter can not be considered as a replication study of the Dutch study, because both studies differ on several points from each other including exposure characteristics, interval between exposure sessions, selection of participants, age of the subjects, and cognitive functions tested.

The question whether the exposure to microwaves might affect cognitive functions is of great importance. Reports of impairment [3, 4] or improvement [5, 6] of cognitive performances in humans are contradicted by findings that no changes occur [7, 8]. Exposure levels of all studies mentioned above are within the ICNIRP guidelines [9]. It is clear that more research is needed to clarify the gap in current knowledge in this field.

1.2. Objective

The nervous system is designed to produce behaviour, so that behavioural analysis is an ultimate assay of neural function. Behaviour is indeed the most sensitive measure of biological effects of microwave effects [10]. Microwaves can interfere with the brain activity and may generate behavioural and cognitive disturbances [11].

In a very recent study Nittby *et al.* [12] found that GSM exposed rats had impaired memory for objects and their temporal order of presentation, compared to the sham-exposed group. This effect was statistically significant ($p = 0.02$).

On the basis of these data, we decided to start a rather small study to assess if a long-term behavioural study should be practically realizable in our facilities at UCL. This is the reason why we have decided for a short-term exposure period of only two months for one part of this experiment. For the other part of the experiment, we utilize rats that have already been exposed for 15 months (*cfr.* Part I). The objective of our behavioural study consists in assessing the possible cognitive effect of microwaves on the *Wistar* albino rat by means of an object recognition task.

1.3. Exposure Protocol and Conditions

The experiment is subdivided into two parts based on age and exposure regime of the rats. The first part was run with two groups of rats, each counting 32 male *Wistar* albino rats, aged 3.5 months and with an average body weight of 350 grams. The groups are composed by randomly distributing the rats by the experimenter. One of these two groups is sham-exposed (group A). The other group (group B) is exposed to a 970-MHz pulse-amplitude-modulated signal, with a pulse repetition frequency of 1 Hz and a duty cycle of 10%. Rats were collectively exposed, two hours a day, five days a week during two consecutive months.

The second part of the experiment was run under identical conditions, with two other groups of *Wistar* albino rats, aged 18 months with an average body weight of 550 grams. One of the groups was sham-exposed (group C). The second half (group D) of the 18-months-old rats has been exposed to a 970-MHz pulse-amplitude-modulated signal, with a pulse repetition frequency of 1 Hz and a duty cycle of 10%. Rats were collectively exposed, two hours a day, seven days a week during 15 months. Periodical blood samplings, five in total, have been performed.

The exposure units are the same as described in Section 3.4 of Part I.

All rats of the four groups were subject to an object recognition task [13]. The procedures for the object recognition task in two-month exposed groups are identical to groups which are exposed during 15 months.

1.4. Field Intensity

Rats are exposed two hours a day to an average power density of 2.2 W/m². A well-established measurement session of the incoming electric field in nearly 80 sampling points inside each exposure unit is meticulously carried out before the start of the experiment. The average electric field strength over all the sampling points equals 28 V/m, which is equivalent with a power density of 2.20 W/m². This value is considered as a low-thermal electromagnetic field. More details on this topic can be found in Section 3.5 of Part I. The recorded power density is totally in line with the ICNIRP reference levels for man [9]. The corresponding SAR value is 0.08 W/kg.

1.5. Behavioural Study Regarding Possible Cognitive Effects of Microwaves

Our approach in evaluating a possible impact of microwaves on the brain function in general and the memory in particular, consists in a behavioural study based on an object recognition task [14]. In the object recognition task, rats are set loose in open space, the test arena, which is an open box of about 1 m^3 made of mat PVC and floor covered with wood chip bedding. The object recognition task consists of a sample phase and a choice phase, each during 3 minutes with a 15-minutes retention interval between the two phases, where after the rat is returned to its cage. The basic measure is the time spent by rats in exploring objects during those two phases. During the sample phase the animals are confronted with two identical, unknown objects. After the first phase, the objects are considered to be familiar, and therefore known to the animal.

In a second phase the rats are placed again in the arena for 3 minutes, but now one of the objects is replaced by a new, unknown object. Animals with

a normal behaviour spend more time exploring a novel object then they do exploring a familiar object. This finding reflects their memory of the familiar object [14]. If an external factor, *e.g.* exposure to low-level microwaves, affects the memory or the exploratory behaviour of the rat, this can be expressed as an impairment in exploratory behaviour. Trough comparison with a sham exposed group, we should be able to see a difference, if any effect is present. The chosen object recognition task offers similarities with human recognition memory [15].

1.6. Object Recognition Task

Rats are habituated to the test arena before the start of the recognition task. Two days prior to the first phase of the recognition task (sample phase), rats are individually allowed to explore the arena (without objects) for 3 minutes. After this the arena is considered as familiar territory [14].

Exploration of an object is defined as directing the nose to the object at a distance of two centimetres or less from the object and/or touching it with the nose.

During the first phase, i.e. the sample phase (T_1), each rat is placed in the test arena with two identical objects (Figure 1.1). At the start of the experiment the rat is placed in the arena with its head turned away from the object. During the 3 following minutes the rat is allowed to explore the arena and the objects while its behaviour is recorded by a camera suspended above the test arena.

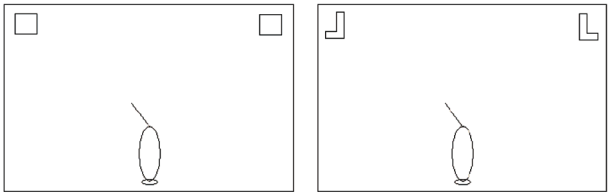

Figure 1.1: Test arena at the start of the sample phase with 2 identical objects.

In the next phase, the choice phase (T_2), one of these objects is randomly removed and replaced by a novel object. This object is different from the objects in T_1 (Figure 1.2). The objects to be discriminated by the rats are assembled from plastic square building blocks. They differ in colour, size, and shape. The test objects are available in multiple copies and are made of a biologically neutral material like plastic. They are not known to have any ethological significance for the rats. Since the animals have never been in contact with these objects before, they are not linked to an external stimulus. After every phase the objects are disinfected, rinsed with water and dried.

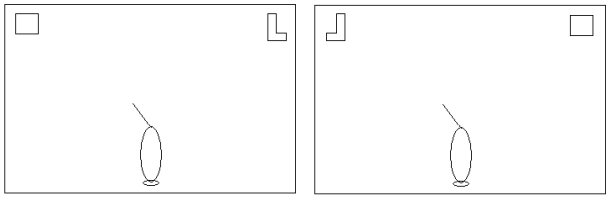

Figure 1.2: Test arena at the start of the choice phase with 2 different objects.

1.7. Analysis of the Time Recordings

The basic measurement in this experiment is the time spent by the rats in exploring the objects during the sample phase T_1 and the choice phase T_2. The parameters we focus on to study the memory effects are calculated with these measurements. The total exploration time e_1 (1.1) in the sample phase

T_1 is the sum of the exploration time of the object that is placed right $T_a(r)$ and the exploration time of the object that is placed left $T_a(l)$.

$$e_1 = T_a(l) + T_a(r) \tag{1.1}$$

The total exploration time e_2 (1.2) in the choice phase T_2 is the sum of the exploration time of the returning object $T_{a'}$ and the exploration time of the new object T_b.

$$e_2 = T_{a'} + T_b \tag{1.2}$$

The habituation index h_1 (1.3) is the difference between the average total exploration times during the two phases. It is an indicator for the habituation of the rat between the two phases. It represents the amount of time that the rats spend more in T_1 exploring the objects then they do in T_2.

$$h_1 = e_1 - e_2 \tag{1.3}$$

The habituation index h_2 (1.4) is the difference between the average exploration time in T_1 and the exploration time of the returning object in T_2. This is the average habituation for the familiar object.

$$h_2 = \frac{e_1}{2} - T_{a'} \tag{1.4}$$

The discrimination index d_1 (1.5) is the difference in time spent exploring the two objects in the choice phase. This is actually a representation of the amount of time the animal spends at exploring the unknown object more than it does at exploring the returning object during T_2.

$$d_1 = T_b - T_{a'} \tag{1.5}$$

The discrimination ratio d_2 (1.6) is the discrimination index d_1 divided by the total time spent exploring the two objects in the choice phase. It gives the time that the animals explore the unknown object more then they explore the familiar one, relative to the total exploration time during the choice phase; so it is independent of the total time of exploration activity.

$$d_2 = \frac{d_1}{e_2} \tag{1.6}$$

The discrimination index d_3 (1.7) reflects a combination of the habituation and discrimination.

$$d_3 = h_2 + d_1 \tag{1.7}$$

Analysis is performed on these measurements. First, in each group apart (exposed and sham-exposed), the time an animal spends exploring the different objects is compared to check if the animals of one and the same group do not make the distinction between two identical objects in the first phase and, to check if they do make this distinction in the second phase (*intra*group comparison). The null hypothesis in both phases is that the average exploration time of the objects is the same. The comparing of data is done by a paired samples t-test.

To test if there is a statistically significant difference in behaviour between exposed animals and sham-exposed animals the data of the several behavioural indices of the two different groups are compared. By means of an independent samples t-test, indices of exposed animals are compared with indices of sham-exposed animals (intergroup comparison). The null hypothesis is that the average value of the indices is equal. The null hypothesis is accepted if $p > 0.05$.

1.8. Results

1.8.1. Animals Exposed During 2 Months

Comparison of the time measurements in the two phases for the sham-exposed group (A) reveals that the animals show no preference for one of the identical objects in T_1 (T_a(l) = 8.53 s; T_a(r) = 8.87 s; p = 0.61). They do make the distinction between the familiar and the new object in T_2, there is a significant difference in average exploration time of the two different objects ($T_{a'}$ = 5.04 s; T_b = 6.73 s; p = 0,009). The exposed group (B) shows a similar behaviour. There is no statistical difference between the exploration behaviour of the identical objects (T_a(l) = 10.09 s; T_a(r) = 10.03 s; p = 0.91), but they do spend more time exploring an unfamiliar object ($T_{a'}$ = 4.86 s; T_b = 6.54 s; p = 0.04) (Table 1.1).

	Group A	**Group B**
T_a(l) vs.T_a(r)	0.61	0.91
$T_{a'}$ vs.T_b	0.009	0.04

Table 1.1: p-values when comparing the exploration times in sample phase *vs.* choice phase for group A and B.

If we compare the several indices between group A and B, there are apparently no differences (Table 1.2). The average total exploration time e_1 during the sample phase is 17.40 s for the sham exposed group and 20.12 s for the exposed group. There is no statistically significant difference between the average total exploration times in T_1 (p = 0.24). The average total exploration time e_2 during the choice phase is 11.77 s for the sham exposed group and 11.40 s for the exposed group. This difference is not statistically significant (p = 0.85). The habituation index h_2 is 3.67 s and 5.35 s for the

sham exposed group and the exposed group, respectively. None of these results are statistically significant ($p = 0.25$ for h_1 and $p = 0.28$ for h_2).

indices	Group A	Group B	p
$\mathbf{e_1}$	17.40	20.12	0.24
$\mathbf{e_2}$	11.77	11.40	0.85
$\mathbf{h_1}$	5.64	9.01	0.25
$\mathbf{h_2}$	3.67	5.35	0.28
$\mathbf{d_1}$	1.70	1.69	0.99
$\mathbf{d_2}$	0.15	0.22	0.50
$\mathbf{d_3}$	5.36	7.04	0.44

Table 1.2: Average values of the behavioural indices for the exposed (B) and sham-exposed (A) group (exposure period: two months).

After comparison of the discrimination indices there is no statistical difference relative to total exploration time (d_1, $p = 0.99$) and (d_2, $p = 0.50$). Regarding the combination of habituation and discrimination (d_3, $p = 0.44$) there is no difference either between exposed and sham exposed animals during 2 months.

1.8.2. Animals Exposed During 15 Months

Comparison of the two phases for the groups exposed to microwaves during 15 months reveals a different behaviour of the exposed group. During T_1 the two groups show normal behaviour, they have no preference for one of the two identical objects in the sample phase. Both for group C ($T_a(l) = 8.45$ s; $T_a(r) = 6.82$ s; $p = 0.12$) and group D ($T_a(l) = 10.06$ s; $T_a(r) = 12.68$ s; $p = 0.44$) the null hypothesis of equal exploration times is accepted (Table 1.3).

In the second phase, the animals of group D do not make the distinction between a familiar and an unfamiliar object ($T_{a'}$ = 4.58 s; T_b = 4.85 s; p = 0.70). Since p > 0.05 we accept the null hypothesis of an equal average exploration time. From this we can conclude that the animals, exposed for 15 months, do not make a distinction between a familiar and an unfamiliar object. The animals in the sham exposed group C ($T_{a'}$ = 3.73 s; T_b = 5.73 s; p = 0.04) do make this distinction in T_2. Thus, via the *intra*group comparison, we can conclude that the exposed animals show derogatory behaviour which is statistically significant.

	Group C	**Group D**
T_a(l) vs. T_a(r)	0.12	0.44
$T_{a'}$ vs. T_b	0.04	0.70

Table 1.3: p-values when comparing the exploration times in sample phase *vs.* choice phase for group C and group D.

indices	**Group C**	**Group D**	**p**
e_1	15.27	17.20	0.34
e_2	9.10	9.43	0.83
h_1	6.18	7.59	0.52
h_2	3.91	3.93	0.99
d_1	1.65	0.27	0.20
d_2	0.23	0.10	0.30
d_3	5.56	4.39	0.49

Table 1.4: Average values of the behavioural indices for the sham exposed (C) and exposed (D) group (exposure period: 15 months).

We proceed with the analysis of the different indices. The difference in total exploration times for the first phase ($e_{1,C}$ = 15.27 s; $e_{1,D}$ = 17.20 s; p = 0.34) and the second phase ($e_{2,C}$ = 9.10 s; $e_{2,D}$ = 9.43 s; p = 0.83) is not statistically significant in the two groups.

Neither the indices of habituation, h_1 ($h_{1,C}$ = 6.18 s; $h_{1,D}$ = 7.59 s; p = 0.52) and h_2 ($h_{2,C}$ = 3.91 s; $h_{2,D}$ = 3.93 s; p = 0.99), are significantly. The discrimination index d_1 features a big difference between exposed and sham exposed group ($d_{1,C}$ = 1.65 s; $d_{1,D}$ = 0.27 s; p = 0.20), d_2 ($d_{2,C}$ = 0.23; $d_{2,D}$ = 0.10; p = 0.30), d_3 ($d_{3,C}$ = 5.56 s; $d_{3,D}$ = 4.39 s; p = 0.49) (Table 1.4). According to the statistical analysis, the difference is not significant. A more sophisticated statistical analysis and replication of the object recognition task might bring more clear results.

1.9. Conclusion

Two groups of 32 rats have been exposed to low-level microwaves in each population. These populations are subject to an object recognition task after 2 and 15 months of exposure, respectively. After two months of exposure the animals show normal exploratory behaviour. There is no statistical difference that can be measured with an object recognition task. The rats that are exposed for 15 months do show derogatory behaviour. They do not make the distinction between a familiar object and an unknown object during the choice phase (T_2). A hypothesis for this derogatory behaviour is related to the impairment of a part of the rat's memory; in that further research is recommended, especially concerning the long-term effects. Some reports suggest that microwave exposure may act on the hypothalamic-pituitary-adrenocortical axis activity and influence the corticosterone and ACTH (Adrenocorticotropic hormone) levels in the peripheral blood. Such effects may interfere with memory performance [14].

However small an effect may be, on the scale of the world population it could still have a big impact. Therefore it is necessary and justified to do further research and a replication of this study. The study will be duplicated in the next few months and the obtained result will be compared in view of the results of the present study.

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Chapter 2 Mortality

In our experiment, four populations of rats have been exposed to different frequencies and exposure modes for 21 months, sham-exposed group included. In total, 93 *Wistar* albino rats have been subject to microwaves with a carrier frequency of 970 MHz CW, 970 MHz PW and 9.70 GHz CW. 31 rats have been sham-exposed.

In epidemiological studies, the effect of an agent on mortality is one of the first parameters to investigate. The mortality rate is the rate at which the population is dying.

2.1. Mortality Rate after 21 Months of Microwave Exposure

We calculated the mortality rate in all the exposed groups compared to the sham-exposed group at the end of the exposure period, *i.e.* after 21 months of daily exposure. The mortality rate is displayed in Figure 2.1, 2.2 and Table 2.1. Rats that have been subject to early euthanasy for ethical reasons have not been taken into consideration.

Parallelly, a longitudinal study, which takes into account the mortality rate along the entire exposure period, has been carried out further in this chapter.

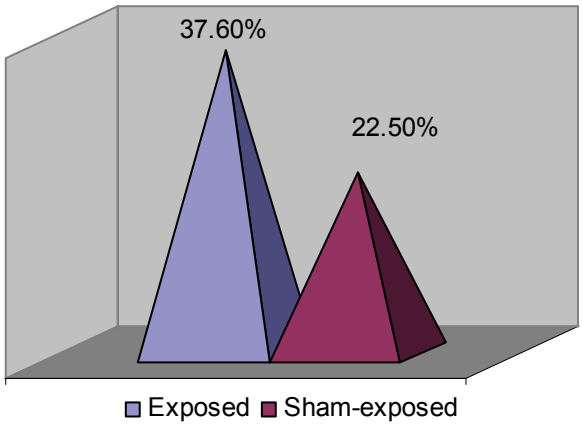

Figure 2.1: Mortality rate in exposed *vs*. sham-exposed rats after a 21-month exposure.

In a first analysis, all the microwave-exposed groups are considered together. It can be seen in Table 2.1 that the mortality rate is higher in the exposed groups than in the sham-exposed group: 37.6% *vs*. 22.5%, respectively.

When differentiating between the three exposed groups, the highest mortality rate is observed in the 970-MHz CW group (38.7%), and the 970-MHz PW group (38.7%) as displayed in Table 2.2. This represents an increase of 72% compared to the sham-exposed rats. The mortality percentage in the 9.70-GHz CW population is 35.5% which is 58% higher than in the sham-exposed group.

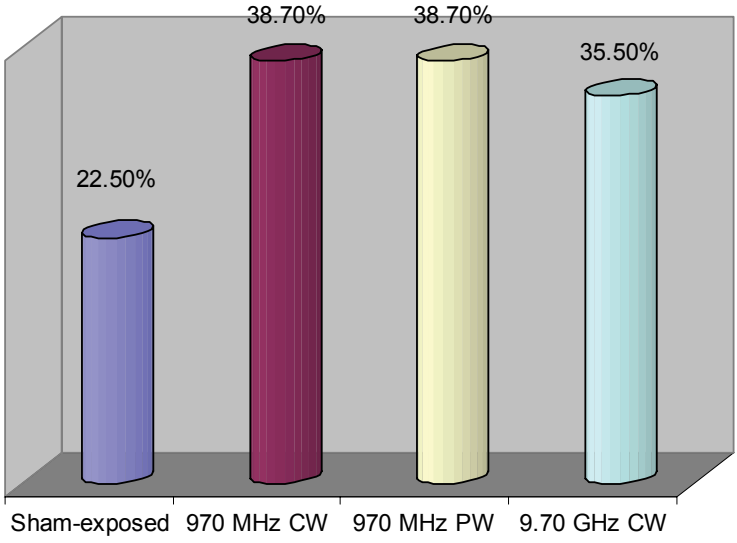

Figure 2.2. Mortality rate in exposed *vs*. sham-exposed groups after a 21-month exposure.

The 22.5% mortality rate for non-exposed rats at the age of 25 months (4 months at the start plus 21 months) is normal (Table 2.1). In the same table, one can observe that a 37.6% mortality rate – this is the mortality rate regarding the 21-months exposed rats in our experiment – is rather high.

	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%
∑ Exposed	58	35	37.6%	62.4%

Table 2.1: Mortality rate in the four exposed groups after a 21-month exposure.

All dead rats are presently submitted to an anatomopathological investigation. This will take quite a time. A sample of the exposed populations has already been analysed. This is described in Part IV.

2.2. Comparing Mortality in Exposed Groups of Rats with Standard Mortality

In our study, we used the *Wistar Han* rat. The Charles River Laboratories performed many tests on survival rates related to this type of rat. Based on 10 longevity studies run during 24 months, typical survival rate data have been obtained for male rats [1]. These are shown in Table 2.2.

The mean survival rate for male *Han* rats after a 24-month period is 78.8%. The worst prognosis is 65.5% and the highest survival rate is 87.3%.

Study Identification	1	2	3	4	5	6	7	8	9	10
Total Number on Study	55	55	55	55	55	55	55	55	55	60
Number of survival	43	47	41	45	44	42	48	46	36	45
Survival%	78.2	85.5	74.5	81.8	80.0	76.4	87.3	83.6	65.5	75.0
Mortality%	21.8	14.5	25.5	18.2	20.0	23.6	12.7	16.4	34.5	25.0

Table 2.2: Summary of individual survival study at the age of 24-months (non-exposed reference *Han* rats, Charles River data).

In order to compare rat survival rate in our experiment to the survival rates in the Charles River reference group, we calculated the mortality rate at the age of 24 months in our group of rats. This can be found in Table 2.3.

	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%
∑ Exposed	65	28	30.1%	69.9%

Table 2.3: Mortality rate in exposed *vs*. sham-exposed groups at the age of 24 months.

An 80.7% survival rate in the sham-exposed group is not abnormal according to the data in Table 2.2. A survival rate of 69.9% in the exposed groups is not common as can be noticed in the same table.

Again, regarding the 970-MHz exposure, no difference in mortality rate between continuous and pulsed exposure can be noticed at the age of 24 months (Table 2.3). A mortality rate of 32.3% is about 52.4% higher than what normally could be expected from male *Han* rats, where the mean mortality rate is 21.2%.

The exposed groups (*cfr*. Figure 2.3) show survival rates situated beneath the mean survival rate (78.8%) of non treated *Han* rats (Table 2.2). These findings make it plausible to us to suggest that the exposure could have had an effect on the overall survival of our rats. Mortality is a very unambiguous parameter regarded to the influence of the independent variable.

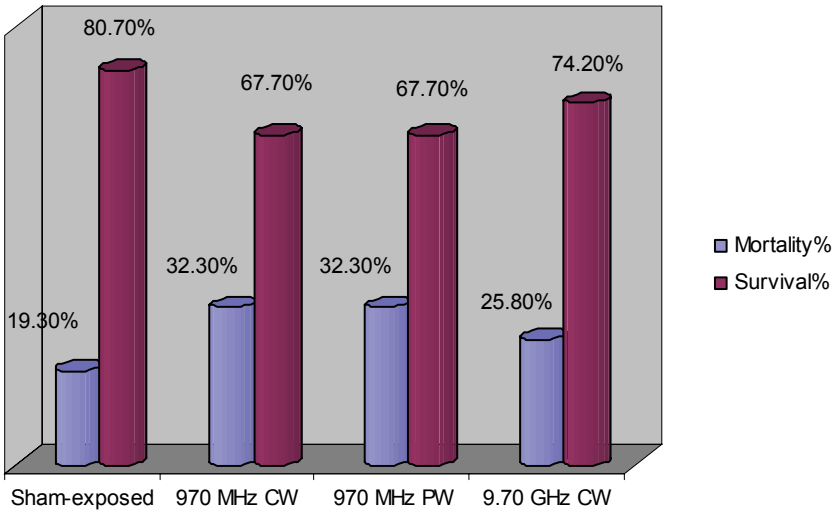

Figure 2.3: Mortality rate in the exposed *vs.* sham-exposed groups at the age of 24 months.

An anatomopathological examination is scheduled to help to interpret this lower than normally expected survival rate. Based on the permanent macroscopic observations we did, and the daily palpations of the rats, we have observed the following symptoms preceding the death: loss of weight associated with infirmity, macroscopic tumours, weakness in the limbs with associated gait abnormality, and breathing problems.

2.3. Mortality Rate after a 3-month Follow-up Post Exposure Period

After 21-month exposure, the surviving rats have been kept alive, but without daily exposure to microwaves. After a 3-month follow-up post-exposure period we determined the mortality rates in exposed and sham-exposed groups (Table 2.4).

After a 3-month follow-up period consecutive to the 21-month exposure period, one can observe the greatest difference between exposed and sham-exposed groups: 29.0% vs. 55.9%. This is a 92.8% increased mortality rate compared to the mortality rate in the sham-exposed group.

	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%
∑ Exposed	41	52	55.9%	44.1%

Table 2.4: Mortality rate in the four exposed groups after a 3-month follow-up period (post 21-month exposure).

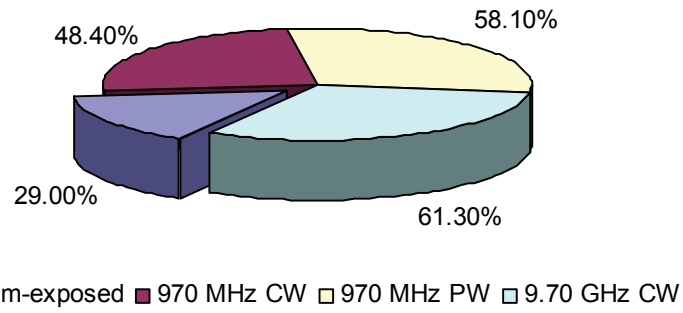

Figure 2.4: Mortality rate in the four exposed groups after a 3-month follow-up period (post 21-month exposure).

When subdividing the whole of the exposed groups in its components, the mortality rate increases from the 970-MHz CW over the 970-MHz PW to the 9.70-GHz CW group as 48.4%, 58.1% and 61.3%, respectively (Figure 2.4).

2.4. Kaplan-Meier Survival Analysis

To consider the mortality in the course of the entire exposure period and to map out the observed survival data, a Kaplan-Meier survival analysis [2] is used to compare mortality in exposed groups with mortality in the sham-exposed group. It is an estimate of the survival function from life-time data and is used to measure the fraction of rats living for a certain amount of time after the experiment has started. On the plot, small vertical lines indicate losses which makes that the survival curve has a stepped pattern. This graphical representation of survival data displays the survival rate during the lifespan of the rats in each group. This can be seen in Figure 2.5.

One statistical procedure that answers the question of whether or not two survival curves are different is the log-rank test [2]. In contrast with some other tests, it is not restricted to two curves. The log-rank test uses a chi-square statistic based on the difference between the observed survival and the survival that would be expected if the curves were not different. The statistical software SPSS 15.0 is used for this calculation.

When comparing the survival curves of sham-exposed rats and 9.70-GHz CW exposed rats after a 3-month follow-up period consecutive to 21 months of exposure, a statistical significant effect ($p = 0.017$) is observed. 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 ($p = 0.349$).

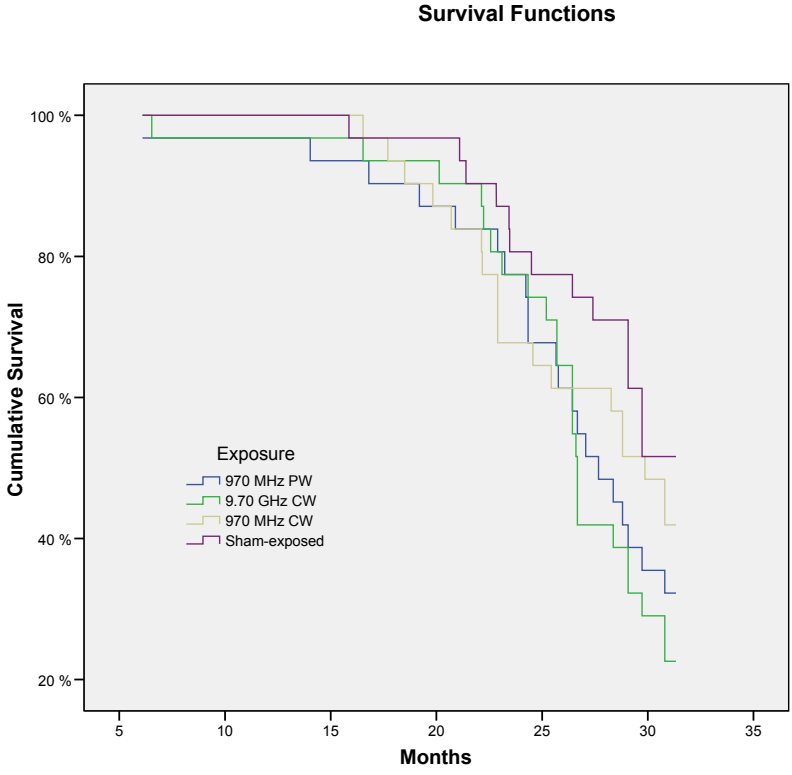

Figure 2.5: Survival curves for 3 exposure types (970 MHz CW, 970 MHz PW, 9.70 GHz CW) compared to the sham-exposed group.

At the end of the experiment, 71% of the sham-exposed rats are still alive, while 38.7%, 41.9%, and 51.6% survived in the 9.70-GHz CW, 970-MHz PW, and 970-MHz CW groups, respectively.

2.5. Discussion

The main objective of this research consists in evaluating if long-term exposure to low-level microwaves is able to produce biological effects on the long run. Naturally increased mortality is an obvious indicator of harmful biological processes taking place in a living organism. An increased mortality rate in microwave exposed groups could be an indication of a firm biological effect. We observed a highly statistically significant increase in mortality in the 9.70-GHz CW exposed group. Although these rats have not been exposed as long as those of the other groups – due to the definitive breakdown of the microwave amplifier – mortality is most expressed in this group both at the end of the experiment as during its course.

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. 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. The fact that we found a statistically significant higher mortality between exposed groups and the sham-exposed group even after forced shutting-down of the microwave exposure during more than the half of the intended exposure period is worthwhile mentioning.

A nearly statistically significant result was found when comparing mortality in the 970-MHz PW group and the sham-exposed group. One should remind that the statistical significance level of 0.05 is arbitrarily chosen. A p-value equalling 0.08 still means that it is unlikely that the result occurred by chance in 92% of the cases. Failing to find evidence that there is a difference

at a significance level of 0.05 does certainly not mean that there is no difference between the exposed groups and the sham-exposed group.

The number of rats used in an experiment is a factor of importance in reaching statistically significant results. On the other hand, observing already significant or nearly significant results in small groups of rats can be an indication of the presence of a persistent effect and may point towards an existing biological effect when larger test populations should be considered.

In literature, there are very few long-term (21 months of *daily* exposure and more) studies reporting survival data in rats. This is a first finding. It is even harder to find mortality studies where rats have been exposed to frequencies beyond 2.45 GHz. Liddle *et al.* [3] found an increased mortality in mice exposed to 2.45 GHz CW, one hour 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 an SAR of 2 W/kg (1 hour per day, 5 days a week, 31 months). Szmigielski *et al.* [4] reported an increased mortality both in mice exposed to a whole body SAR of 2-3 W/kg and 6-8 W/kg (2 hours a day, 6 days a week, 10.5 months; 2.45 GHz). Utteridge *et al.* [5] found no statistically significant difference in mortality between sham-exposed groups and mice exposed to 898.4 MHz GSM exposure (1 hour a day, 5 days a week, 24 months) at whole body SAR of 0.25 W/kg, 1 W/kg, 2 W/kg, and 4 W/kg.

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. Besides, when comparing 24-month old rats in exposed groups, sham-exposed group and ten non-exposed reference groups it can be noticed that mortality rate is always higher in exposed groups (30.1%) than in ten

non-exposed reference groups (mean mortality rate equals 21.2%) and than in the sham-exposed group (19.3%).

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Part IV : Anatomopathology

The aim of the anatomopathological part in our study consists in examining if the exposed rats present more neoplasms or other major tissue lesions compared to the rats in the sham-exposed group. Performing a complete autopsy on each of the 124 rats in the exposed population is quite a lot of work and goes beyond the purpose of this thesis. Though, we took a sample of 19 rats that have been completely analysed as described further in the anatomopathological protocol.

The results of the autopsy are presented in chapter 2. It gives a first impression of the types of histological anomalies that can be encountered in rats that have been subject to a long-term microwave exposure.

Chapter 1 Autopsy Protocol

1.1. Measuring, Description, and Fixation

First we proceed to the macroscopic examination of the rat cadavers: general appearance, weight, measurements, possible presence of lesions,… followed by a description of the condition of the fur.

Biological tissues must undergo a series of treatments to be observed with a light microscope. The process begins by stabilization of the tissue with chemical fixatives. Next, the tissue is made rigid to allow sectioning. Finally, it is stained to provide contrast for visualization in the microscope.

As the rats are observed continuously every day, the cadavers are still fresh when we proceed to the conserving procedure. Each rat cadaver is first dissected, followed by an evisceration and then put in a fixation solution of

formalin 10% (minimum 24 hours). Once fixated, the organs can be preserved for several months if necessary.

The next step consists in weighting and description of the organs and putting them into cassettes. The height, appearance and consistence of the lesions as well as their relation with the healthy neighbouring tissue and the boundaries are reported.

1.2. Cassettes

From each rat 10 or more samples (cerebrum, cerebellum, thymus, digestive tract, stomach, pancreas, heart, lungs, liver, spleen, kidney, adrenals, bladder, and testes) are taken, both of healthy tissue and macroscopic detectable lesions and putting them into cassettes, ready for the dehydration of the tissue samples.

1.3. Dehydration and Substitution

This stage prepares the tissues for inclusion in paraffin, which is realized in an automaton. Tissue water is not miscible with the embedding solutions and must be replaced using a series of alcohols at increasingly higher concentrations [1]. Thus, the inclusion will only succeed if the tissue does not contain either water or an intermediary solvent; therefore the anatomic piece must be totally dehydrated by the use of alcohol before inclusion in the paraffin. This step is followed by alcohol replacement with an intermediate solvent that is miscible with both alcohol and the embedding solution. Because the paraffin is not soluble in alcohol either, the alcohol has to be replaced by toluene.

So a double substitution is proceded.

Dehydratation: 5 minutes per bath

Alcohol 80%

Alcohol 90%

Alcohol 100%

Substitution: 5 minutes per bath

Carbolic toluene first bath

Carbolic toluene second bath

Pure toluene first bath

Pure toluene second bath

1.4. Inclusion in Paraffin

The tissues are included in a mixture of paraffin and plastic polymers. There are different qualities of paraffin, characterised by their melting-point. Paraplast is synthetic paraffin that impregnates well the anatomic pieces. The temperature of the paraffin bath is adjusted in function of there respective melting-point (45°C – 70°C). The duration of the inclusion depends on the size of the anatomic piece. The inclusion takes place in a mould enabling to confect blocs which are sliced in the microtome.

1.5. Slicing the Paraffin

The embedded tissue is cut thin enough to allow a beam of light (or electrons) to pass through. The paraffin bloc containing the tissue is cut into slices of 4 to 5 μm thick.

1.6. Displaying on Slides

The obtained slices are spread out on slides which are dried to ensure a good adhesion to the slide before colouring. We used a standard colouring using a Hematoxyline-Eosine (HE) solution [2]. Hematoxyline colours the cell nuclei dark violet; the eosin colours the cytoplasm pink.

Hemalun acid of Mayer, 15 minutes
Rinse with water, 15 minutes
Eosine
Fuchsine-ponceau (or Ponceau xylidin), 3 minutes
Acetified water, 15 seconds
Phosphomolibdic acid 1%, 10 minutes
Acetified water, 15 seconds
Aniline blue, 2 minutes

1.7. Microscopical Analysis of the Slices

This only colouration is enabling to diagnose the great majority of tumour and non tumour lesions. In some cases specialised analyses are required (special colouration, immunohistochemical examination, in situ hybridization, PCR) to get a complete diagnosis [1].

References

[1] A. S. Pakurar, J. W. Bigbee, *Digital Histology*, Wiley, New Jersey, 2004.

[2] A. Stevens, J. S. Lowe, B. Young, *Wheater's Basic Histopathology*, 4th Edition, Churchill Livingstone, London, 2002.

Chapter 2 Anatomopathological Analysis

2.1. Anatomopathological Analysis of the Samples

Due to practical reasons, we have chosen to examine only 19 samples: two rat cadavers come from the sham-exposed group, seven rats from the 970-MHz CW exposed group, five samples from the 9.70-GHz CW group and five rat cadavers from the 970-MHz PW group. The anatomopathological observations related to each rat are displayed in Tables 2.1 to 2.19.

2.1.1. Sham-exposed Rats

A. Rat R13 of the sham-exposed group

The external and internal examination is summarized in Table 2.1

External examination	
Weight	600.00 g
General aspect	No abnormal macroscopic pathological findings
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.28 g
Cerebellum	0.52 g
Heart	2.62 g
Lungs	4.60 g
Liver	14.40 g
Spleen	0.79 g, normal

Kidneys	3.43 g
Adrenal glands	0.07 g (unilateral)
Digestive tract	Normal
Stomach	Normal
Pancreas	Normal
Testes	3.23 g, normal

Table 2.1: Anatomopathological data of rat R13.

During microscopic examination prominent mucous cells in small intestine (ileum) can be observed.

B. Rat R14 of the sham-exposed group

The external and internal examination is described in Table 2.2.

External examination	
Weight	507.11 g
General aspect	Firm, white lobulated subcutaneous nodule abdomen, 11.18 g
Fur	White – yellow, clean
Internal examination	
Cerebrum	Normal
Cerebellum	Normal
Heart	2.53 g
Lungs	Normal, 3.08 g
Liver	15.75 g
Spleen	Normal, 1.38 g
Kidneys	Bilateral progressive nephropathy, 3.41 g
Adrenal glands	Not retrieved

Digestive tract	Normal
Stomach	Normal
Pancreas	Normal
Testes	Normal, 4.57 g

Table 2.2: Anatomopathological data of rat R14.

The skin nodule presents an infiltrating mass of pleiomorphic spindle and oval cells, with marked anisokaryosis, organized in whorls and interwoven bundles. It can be characterized as a sarcoma. An anisokaryosis is characterized by an unequal size and feature of cell nuclei.

2.1.2. Rats of the 970 MHz CW-group

A. Rat G3 of the 970-MHz CW group

The external and internal examination is described in Table 2.3.

External examination	
Weight	532.00 g
General aspect	Normal
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.74 g
Cerebellum	0.74 g
Heart	3.95 g
Lungs	White pinpoint foci (left lung), 3.10 g
Liver	30.20 g
Spleen	1.44 g
Kidneys	Irregular surface, bilateral, 4.50 g
Adrenal glands	0.19 g

Digestive tract	Normal
Stomach	Normal
Pancreas	Normal
Testes	Right, nodule (3.5 x 2.2 x 1.5 cm), yellowish, 10.53 g (Right testis = 8.85 g)

Table 2.3: Anatomopathological data of rat G3.

A marked diffuse macrovesicular hepatocytic vacuolation (lipidosis) is seen in the liver. Lipidosis is a disturbance in the metabolism of lipids. Kidneys show a marked bilateral, diffuse, chronic, progressive nephropathy which is in concordance with the macroscopic observation of the kidneys.

The unilateral multilobular nodule on the testis is identified as a Leydig cell adenoma.

Leydig cells are clusters of endocrine-secreting cells adjacent to the seminiferous tubules in the testes and producing testosterone. About 10% of these neoplasms act in an aggressive fashion, with local invasion or metastases [1].

B. Rat G4 of the 970-MHz CW group

This rat had a tumour at the right shoulder, which hindered it from moving around.

The rat G4 died after the blood sampling of period 4.

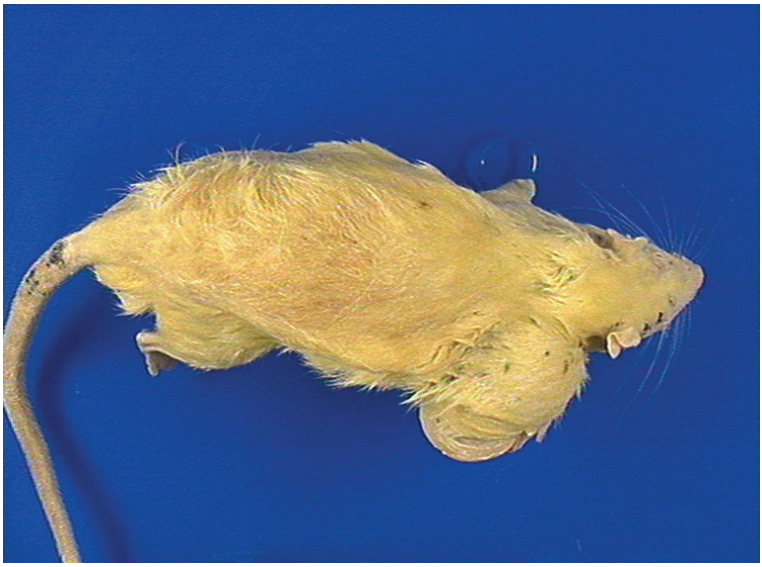

Figure 2.1: Rat G4 (970 MHz CW) with tumour at right shoulder.

The external and internal examination is summarized in Table2.4.

External examination	
Weight	645 g
General aspect	Tumour right shoulder, right side, 44 grams, 4.5 x 2.5 cm. Solid tumour, soft aspect, well delimited. At the section: uniformly white
Fur	White – yellow, clean
Internal examination	
Thymus	Normal
Heart	3.5 g
Lungs	7.0 g
Liver	Normal, 25 g
Spleen	<1 g
Kidneys	Normal, 4 g
Adrenal gland	Normal

Digestive tract	Normal
Bladder	Normal
Testes	Normal, 2.5 g each

Table 2.4: Anatomopathological data of rat G4.

The tumour (Figure 2.2: cross-section, Figure 2.3: histological examination) is identified as a lipoma, a common benign tumour composed of fatty tissue. Lipomas are the most common soft tissue tumour [1].

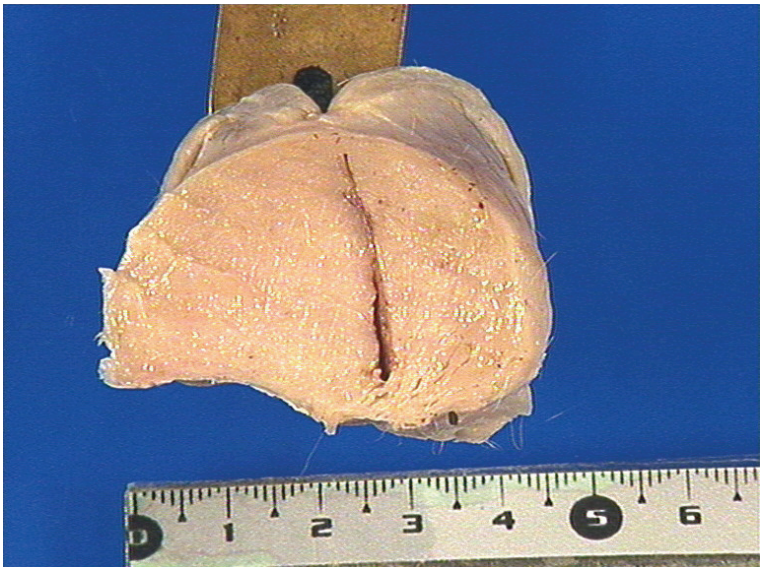

Figure 2.2: Cross-section of the tumour of rat G4 (970-MHz CW group).

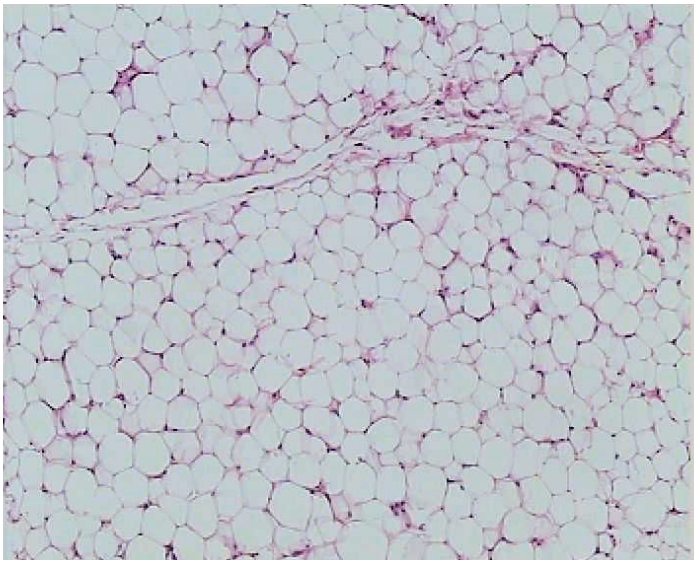

Figure 2.3: Tumour of the right shoulder (rat G4, 970-MHz CW group) presents the characteristics of a lipoma.

C. Rat G7 of the 970-MHz CW group

The external and internal examination is described in Table 2.5.

External examination	
Weight	524.00 g
General aspect	Normal
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.72 g
Cerebellum	0.67 g
Heart	3.69 g
Lungs	6.11 g
Liver	Mottled, small white foci, 29.10 g
Spleen	1.10 g

Kidneys	Irregular surface, 7.10 g
Adrenal glands	0.14 g
Digestive tract	Normal
Stomach	Normal
Pancreas	Normal
Testes	2.76 g

Table 2.5: Anatomopathological data of rat G7.

Microscopically, the liver presents a marked multifocal macrovesicular hepatocellular vacuolation (lipidosis) what explains the macroscopic white small foci. The pancreas shows a marked necrotizing arteritis (mesenterial arteries). Further, a marked bilateral, diffuse, chronic, progressive nephropathy is observed. Microscopic analysis of testis tissue reveals a unilateral Leydig cell adenoom and a moderate unilateral multifocal Leydig cell hyperplasia.

D. Rat G16 of the 970-MHz CW group

The external and internal examination is described in Table 2.6.

External examination	
Weight	544.39 g
General aspect	Swollen testes
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.49 g
Cerebellum	0.59 g
Mesenterium	Multiple proliferations caudal abdominal cavity, pelvic cavity
Heart	Atrial thrombosis, 5.66 g
Lungs	White foci, 1-3 mm, all lobes, 3.11 g

Liver	Mottled, 26.90 g
Spleen	Normal, 1.56 g
Kidneys	Irregular surface, bilateral, 4.53 g
Adrenal glands	0.18 g
Digestive tract	Normal
Stomach	Normal
Pancreas	Atrophic
Testes	Left testis enlarged, right testis white areas at cut surface, 14.68 g

Table 2.6: Anatomopathological data of rat G16.

The mottled macroscopic feature of the liver can be explained by the microscopical observed macrovesicular hepatocellular vacuolation (lipidosis). The pancreas presents a diffuse, marked, lipomatous atrophy. The white foci situated on the lungs are probably caused by a moderate, multifocal, alveolar histiocytosis. Kidneys show a moderate, bilateral, diffuse, chronic, progressive nephropathy and a unilateral cyst. Testes show bilateral Leydig cell adenoma and mesothelioma (mesorchium: fold between testis and epididymis). The mesothelium is a simple squamous epithelium [2]. The serous membrane or serosa lines the perimeter of all internal body cavities that do not open to the exterior, such as peritoneal cavity. It covers and forms the outer layer of any organs that protrude into those cavities, such as stomach and jejunum (peritoneum), lungs (pleura), and heart (pericardium). This covering constitutes the visceral layer of these cavities.

E. Rat G19 of the 970-MHz CW group

The external and internal examination is described in Table 2.7.

External examination	
Weight	412.41 g
General aspect	Thinner than other rats. Swollen mesenteric lymph nodes, dark red
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.44 g
Cerebellum	0.73 g
Heart	Atrial thrombosis, 5.66 g
Lungs	2.92 g
Liver	Multiple red foci, 1-3 mm to small nodules, 16.50 g
Spleen	Mottled, 1.06 g
Kidneys	Irregular surface, bilateral, 3.15 g
Adrenal glands	0.19 g
Digestive tract	Normal
Stomach	Normal
Pancreas	Atrophic
Testes	2.59 g

Table 2.7: Anatomopathological data of rat G19.

A slight multifocal fibrosis is observed in spleen slices; the fibrosis can be related to the mottled macroscopic feature of the spleen. Microscopic analysis of liver tissues reveals a moderate multifocal fibrosis and a moderate multifocal necrosis and haemorrhage. Kidneys present a moderate bilateral, diffuse, chronic, progressive nephropathy. In testis, a unilateral

focal Leydig cell hyperplasia and a unilateral Leydig cell adenoma is observed.

F. Rat G20 of the 970-MHz CW group

The external and internal examination is described in Table 2.8.

External examination	
Weight	680.70 g
General aspect	Normal
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.55 g
Cerebellum	0.50 g
Heart	Atrial thrombosis, 4.06 g
Lungs	Enlarged, irregular colour (white-grey), 6.17 g
Liver	Mottled, white-yellow discoloration, 12.78 g
Spleen	Autolysis, 0.82 g
Kidneys	Irregular surface, bilateral, 3.20 g
Adrenal glands	0.16 g
Digestive tract	Normal
Stomach	Normal
Pancreas	Atrophic
Testes	Unilateral, irregular yellow colour, 2.82 g

Table 2.8: Anatomopathological data of rat G20.

Rat G20 presents a severe, diffuse, bilateral lung emphysema and a marked multilateral alveolar oedema. Liver shows moderate, diffuse, hepatocellular atrophy and severe, diffuse, macrovesicular, hepatocellular vacuolation (lipidosis). The irregular surface of the kidneys is caused by a bilateral,

diffuse, chronic, progressive nephropathy. Testes show unilateral Leydig cell adenoma.

G. Rat G21 of the 970-MHz CW group

Rat G21 suffered from a tumour on its left shoulder. The tumour hindered the rat from freely moving around. Rat G21 died after the fourth blood sampling, *i.e.* after 14 months of exposure.

In Table 2.9 we describe the external and internal examination of the rat.

External examination	
Weight	700 g
General aspect	Subcutaneous tumour left shoulder, 47.5 grams, 11.0 x 7.0 cm. Solid tumour, soft aspect, well delimited. At the section: crossed with several fibrous cords.
Fur	Yellow, clean
Internal examination	
Thymus	Small
Heart	Normal, 2.0 g
Lungs	Normal, except of the small white spots (pleural side), 4.5 g
Liver	Normal, 12 g
Spleen	<1 g
Kidneys	Normal, 4 g
Adrenal gland	Normal
Digestive tract	Normal; presence of blood in stomach
Bladder	Normal
Testes	Normal, 2.5 g each

Table 2.9: Anatomopathological data of rat G21.

The removal of the tumour (Figure 2.5, cross-section Figure 2.6) left a well-defined hole in the shoulder (Figure 2.4).

After examination, the tumour was identified as a fibrosarcoma, a malignant tumour derived from fibrous connective tissue [3]. This tumour is characterized by immature proliferating fibroblasts or undifferentiated anaplastic spindle cells.

Figure 2.4: Rat G21 after removal of the tumour. The head was also removed for examination of the brains.

Figure 2.5: Tumour (fibrosarcoma) from left shoulder of rat G21.

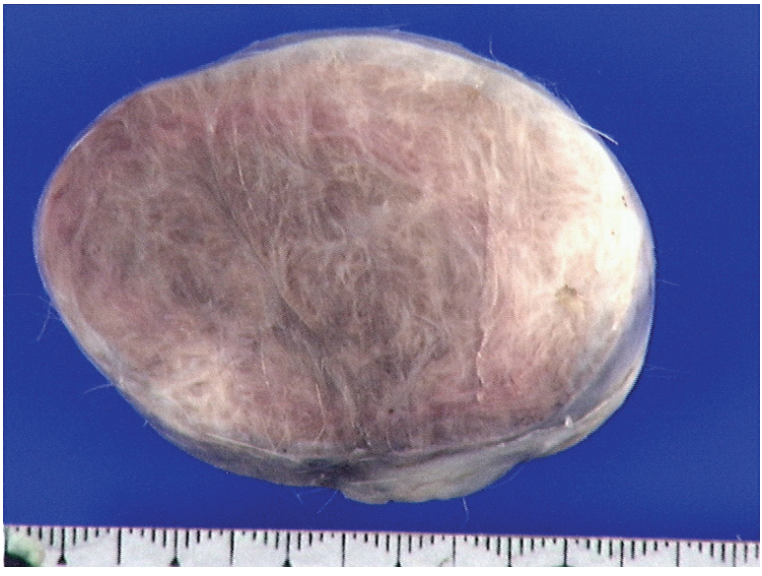

Figure 2.6: Cross-section of tumour from left shoulder of rat G21.

2.1.3. Rats of the 9.70 MHz CW-group

A. Rat B2 of the 9.70-GHz CW group

The external and internal examination is described in Table 2.10.

External examination	
Weight	582.76 g
General aspect	Nodule (3.0 x 2.0 x 1.5 cm) beneath left eye; soft, white cystic area infiltrating the maxillla
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.56 g
Cerebellum	0.50 g
Retropharyngeal lymph node	Swollen, left side
Heart	2.28 g
Lungs	3.66 g
Liver	Irregular white-yellow discoloration with red foci, 17.54 g
Spleen	Normal
Kidneys	3.00 g
Adrenal glands	0.18 g
Digestive tract	Normal
Stomach	Normal
Pancreas	Normal
Testes	3.07 g

Table 2.10: Anatomopathological data of rat B2.

Microscopic examination of the liver shows severe, diffuse hyperaemia which may explain the macroscopically observed red foci. The liver further demonstrates a moderate multifocal macrovesicular hepatocytic vacuolation (lipidosis). A moderate, diffuse, bilateral hyperaemia is seen in the adrenals. The macroscopically observed skin nodule is a squamous cell carcinoma (malign). Squamous cells are much wider than tall, resembling a "fried egg" [3]. The nucleus is highly flattened. Squamous cell carcinomas may arise in any site of native stratified squamous epithelium, for example skin. The infiltrating mass consists of proliferating dysplastic keratinocytes with marked nuclear and cytological pleiomorfism characterized by a high mitotic index. The swelling in the retropharyngeal lymph node is identified as a metastasis of a squamous cell carcinoma.

B. Rat B6 of the 9.70-GHz CW group

The external and internal examination is described in Table 2.4.

External examination	
Weight	352.25 g
General aspect	Nodule (3.5 x 2.5 x 2.0 cm) on foreleg, 13.93 g
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.35 g
Cerebellum	0.60 g
Heart	2.85 g
Lungs	5.61 g
Liver	Yellow discoloration, mottled, 12.25 g
Spleen	0.37 g
Mesenteric lymph nodus	Red discoloration
Kidneys	Severe irregular surface, bilateral, 6.18 g

Adrenal glands	Unilateral, swollen, red, 0.25 g (swollen gland: 0.18 g)
Digestive tract	Normal
Stomach	Normal
Pancreas	Normal
Testes	1.24 g

Table 2.11: Anatomopathological data of rat B6.

At the microscopic examination, prominent mucous cells could be detected at the ileum. A slight diffuse macrovesicular hepatocellular vacuolation (lipidosis) can explain the macroscopic yellow discoloration of the liver. The lipidosis is defined as a disturbance in the lipid metabolism. In the exocrine pancreas an acinar (or alveolar) cell atrophy is observed. Both kidneys present a bilateral, diffuse, chronic, progressive nephropathy. In the adrenals, a unilateral massive haemorrhage with cortical necrosis is encountered, which can be at the origin of the observed weight loss. The heart presents a marked, diffuse, and chronic myocarditis, especially the left ventricle. The tumour on the foreleg is malignant and presents an encapsulated, infiltrating pleiomorphic cellular mass of oval to round cells with marked anisokaryosis and a high mitotic index. Some central areas of necrosis and multifocal lymphocytic infiltrates can be distinguished. In order to further identify this malignant tumour immunohistochemical tests are necessary.

C. Rat B21 of the 9.70-GHz CW group

The external and internal examination is described in Table 2.12.

External examination	
Weight	553.90 g
General aspect	Thinner then other rats
Fur	White – yellow, clean

Internal examination	
Cerebrum	1.55 g
Cerebellum	0.62 g
Heart	Normal, 2.77 g
Lungs	3.45 g
Liver	16.11 g
Spleen	Normal, 0.76 g
Kidneys	Several small cortical cysts, 3.43 g
Adrenal glands	0.13 g
Digestive tract	Normal
Stomach	Normal
Pancreas	Abnormal
Testes	Normal

Table 2.12: Anatomopathological data of rat B21.

The microscopic analysis of the pancreas reveals an adenocarcinoma, a small area of displastic tubules with strong stromal reaction. An adrenocarcinoma is a malignant neoplasma of epithelial tissue in glandular tissue [4]. Kidneys present minimal bilateral diffuse chronic progressive nephropathy. It consists of a glomerular sclerosis and a thickening of Bowman's capsule and may be associated with tubular atrophy, hypertrophy, and hyperplasia. A diffuse bilateral cortical vacuolation is observed in the adrenals.

D. Rat B27 of the 9.70-GHz CW group

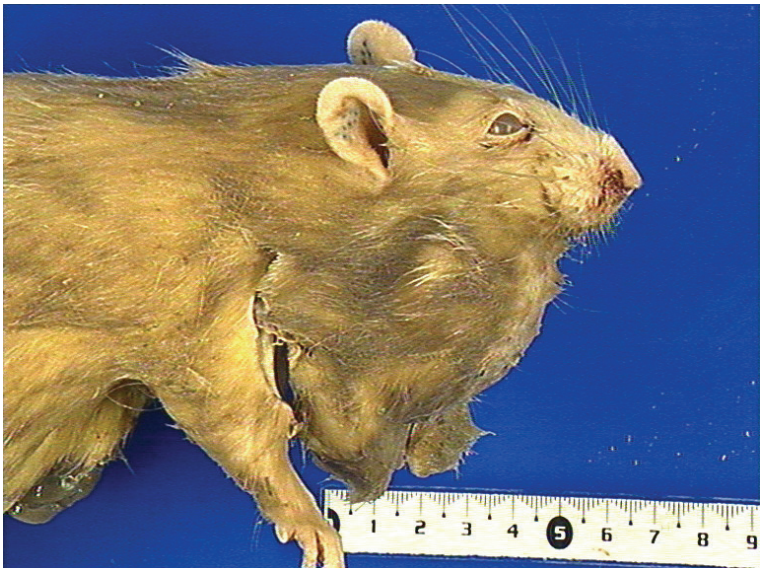

Figure 2.7: Rat B27 of the 9.70-GHz CW group with cervical tumour.

Rat B27 died after the fifth blood sampling.

The examination of the rat is described in Table 2.13.

External examination	
Weight	840 g
General aspect	Cervical tumour, right side, 41 grams, ovoid, 5 x 3 x 2 cm. Solid tumour, polylobular, well delimited. At the section: white and beige tissue and some haemorrhagic zones
Fur	White – yellow, clean
Internal examination	
Thymus	Normal
Heart	3.5 g, left ventricle rather thick
Lungs	7.5 g, some white zones of 2-3mm at the base

Liver	Normal, 27 g
Spleen	2 g
Kidneys	Normal, 5 g
Adrenal gland	Normal
Digestive tract	Normal
Bladder	Normal
Testes	Normal, 2.5 g each

Table 2.13: Anatomopathological data of rat B27.

The tumour (Figure 2.8, cross-section in Figure 2.9 and histological examination in Figure 2.10) is subdivided in multiple lobes by conjunctive tissue. The tumour is composed of epithelial cells, relatively poor differentiated. Several necrotic zones, sometimes haemorrhagic with infiltrates of polynuclear white blood cells. Additional tests are needed to entirely identify the tumour.

Figure 2.8: Cervical tumour polylobular aspect.

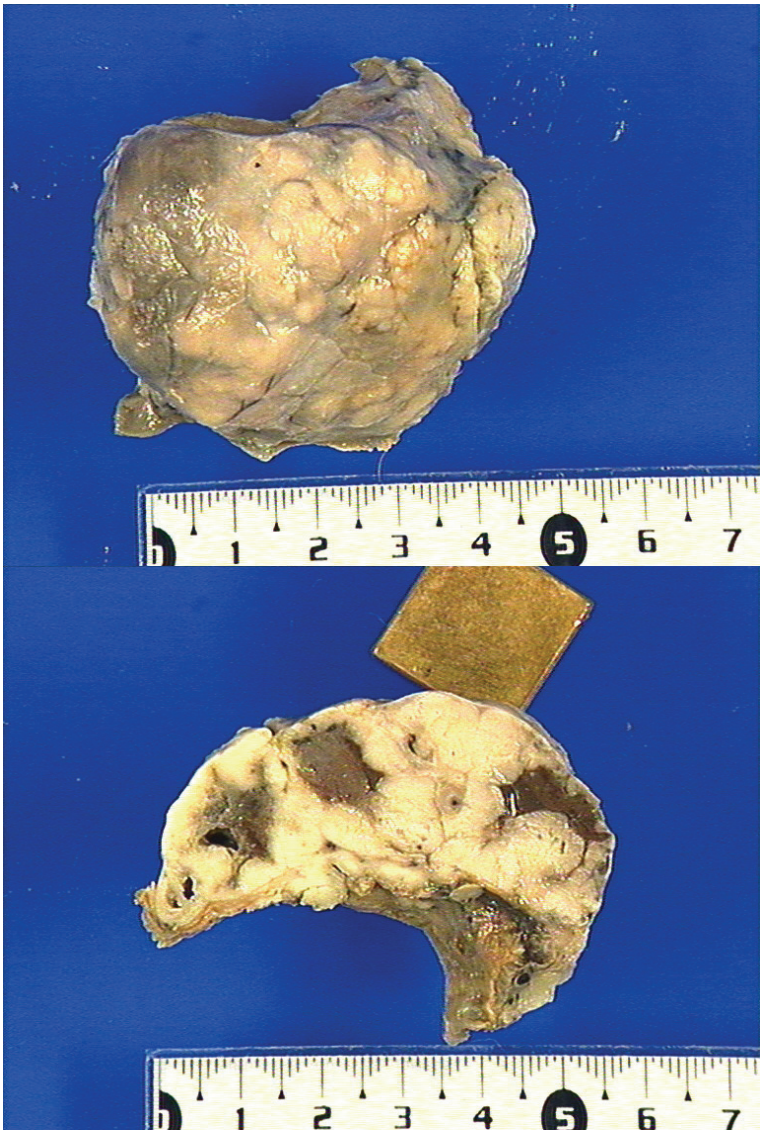

Figure 2.9: Cross-section of the cervical tumour.

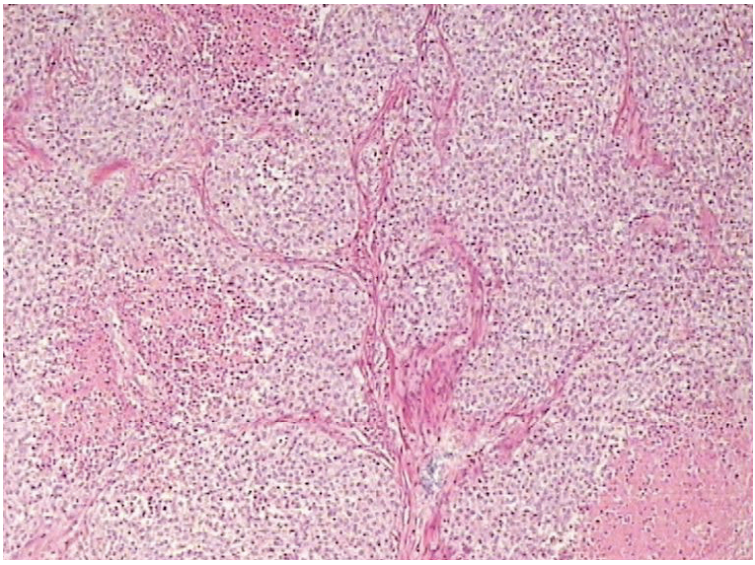

Figure 2.10: Histological examination: the tumour.

E. Rat B28 of the 9.70-GHz CW group

The external and internal examination is described in Table 2.14.

External examination	
Weight	793.71 g
General aspect	Skin nodule (8.0 x 6.0x 5.0 cm), 101.34 g, well demarcated, ventral abdomen, right
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.71 g
Cerebellum	0.61 g
Heart	Normal, 2.94 g
Lungs	3.41 g
Liver	Yellow discoloration with red area, mottled, 26.82 g
Spleen	1.11 g

Kidneys	4.39 g
Adrenal glands	Not retrieved
Digestive tract	Normal
Stomach	Normal
Pancreas	Multifocal area of fat tissue infiltration
Testes	Normal, 4.13 g

Table 2.14: Anatomopathological data of rat B28.

The skin nodule shows the characteristics of a sarcoma which is a malignant tumour. An infiltrating mass of pleiomorphic spindle and oval cells with marked anisokaryosis, organized in whorls and interwoven bundles can be macroscopically observed in the tumour slices.

2.1.4. Rats of the 970-MHz PW group

A. Rat Y1 of the 970-MHz PW group

Rat Y1 limped because of a tumour at its right hind limb. Rat Y1 died before the fifth blood sampling.

The external and internal examination is described in Table 2.15.

External examination	
Weight	845 g
General aspect	Voluminous tumour right thigh, 240 g, 7.0 x 8.0 cm. At the section: crossed with several fibrous cords
Fur	Yellow, clean
Internal examination	
Thymus	Normal
Heart	Normal, 3.0 g
Lungs	Normal, 4.5 g

Liver	Normal, 20.5 g
Spleen	<1 g
Kidneys	4.5 g
Adrenal gland	<1 g
Digestive tract	Normal
Bladder	Normal
Testes	Normal, 2.5 g each

Table 2.15: Anatomopathological data of rat Y1.

The tumour (Figure 2.11 and cross-section Figure 2.12) was identified as a fibrosarcoma [3]. Densely packed neoplastic cells are arranged in long fascicles. Spindle sarcomatous cells show hyperchromasia and mitotic figures. The cells of the tumour (anisocytosis, Figure 2.13) as well as the nuclei (anisonucleosis, Figure 2.14) are abnormal unequal in size.

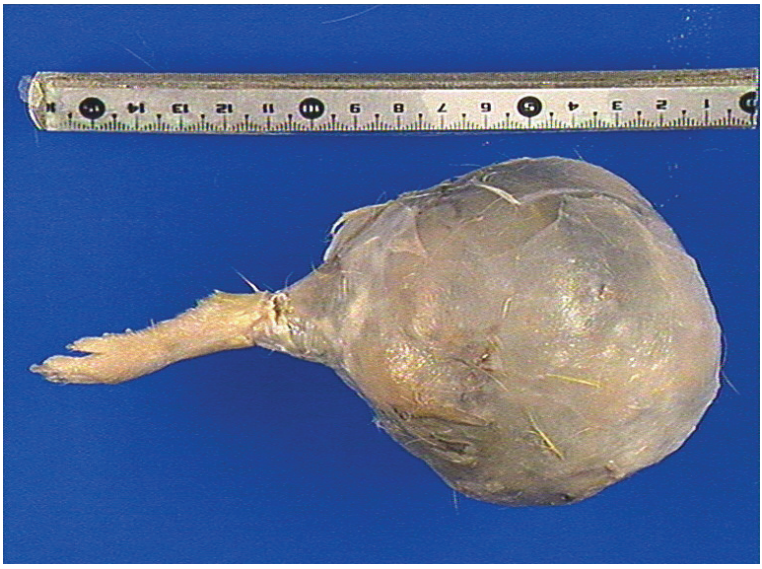

Figure 2.11: Resection of right hind limb with tumour (Rat Y1).

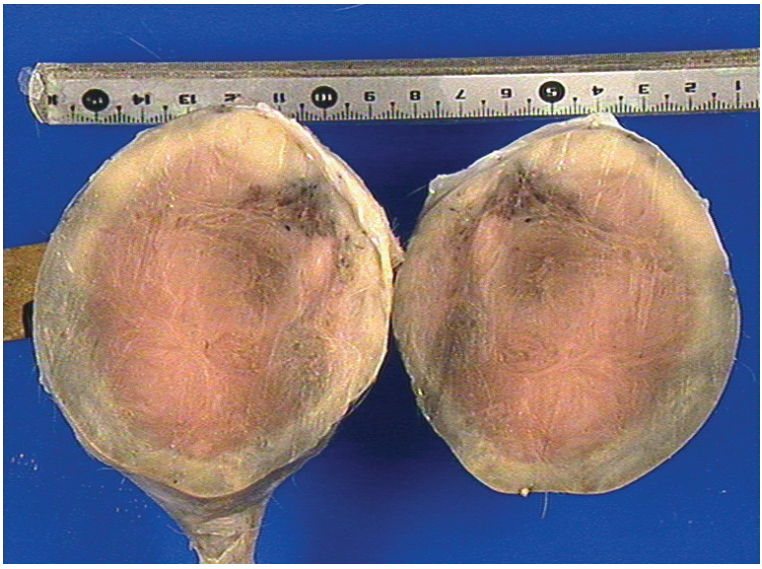

Figure 2.12: Cross-section of tumour (Rat Y1, 970 MHz PW).

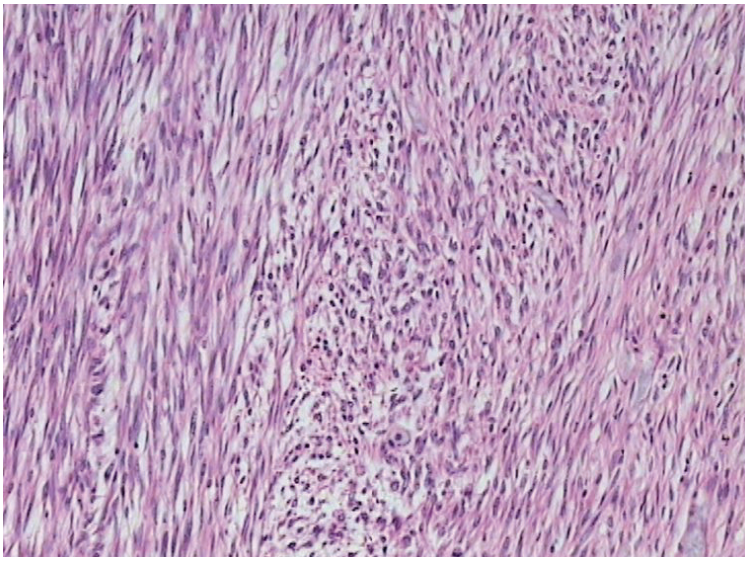

Figure 2.13: Histological slice of tumour of type fibrosarcoma anisocytosis (Rat Y1, 970 MHz PW).

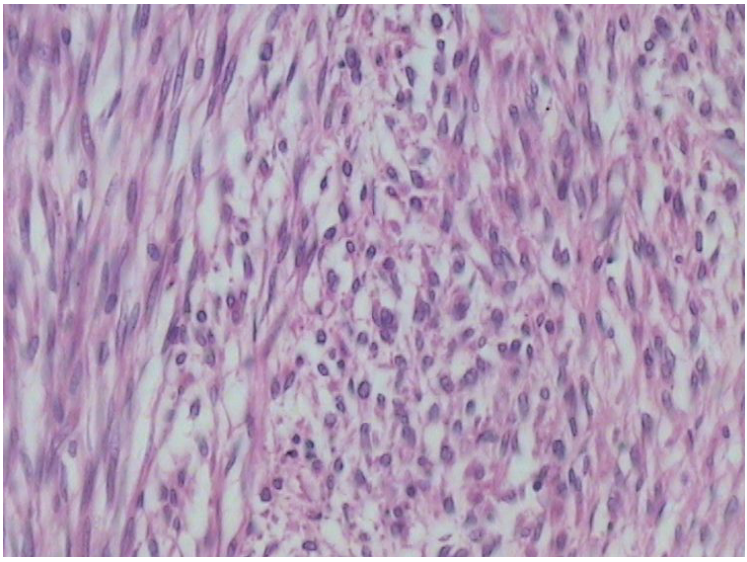

Figure 2.14: Histological slice of tumour of type fibrosarcoma, anisonucleosis (Rat Y1, 970 MHz PW).

B. Rat Y3 of the 970-MHz PW group

Rat Y3 suffered from a cervical tumour on its left. Its weight was also decreased compared to the group average.It died before the blood samplings of the 4th period.

The description of the external and internal examination is given in Table 2.16.

External examination	
Weight	500 g
General aspect	Thinner than other rats Cervical tumour left shoulder; well delimited
Fur	Yellow

Internal examination	
Thymus	Normal
Heart	Normal, 2.0 g
Lungs	Normal, 10 g
Liver	16 g
Spleen	<1 g
Kidneys	4 g
Adrenal gland	-
Digestive tract	Normal
Bladder	Normal
Testes	Normal

Table 2.16: Anatomopathological data of rat Y3.

The tumour (figure 2.15, cross-section figure 2.16, histological preparation figure 2.17) has been identified as a keratoacanthoma [4]. A keratoacanthoma is considered as a benign epithelial tumour. It develops rather rapidly and is relatively common found [5].

It is characterised by deep bulbous lobules of keratinizing well differentiated squamous epithelium with central keratin filled crater.

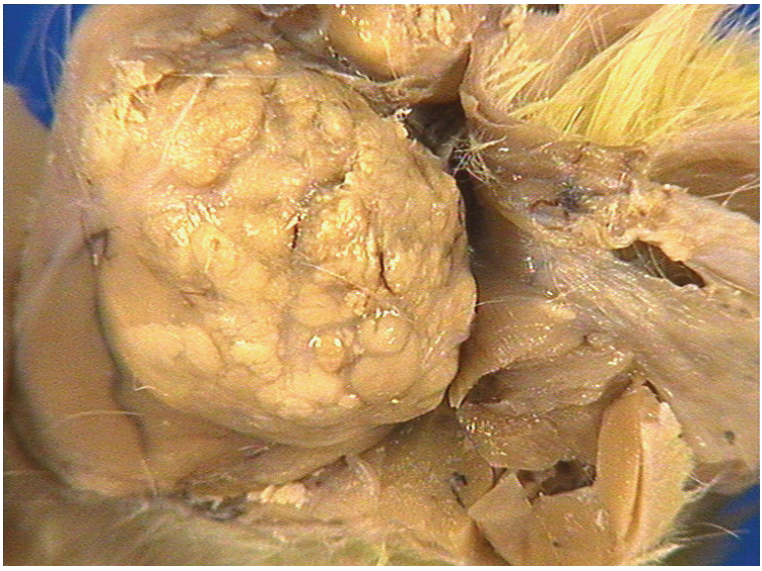

Figure 2.15: Cervical tumour of rat Y3, composed of multiple lobes.

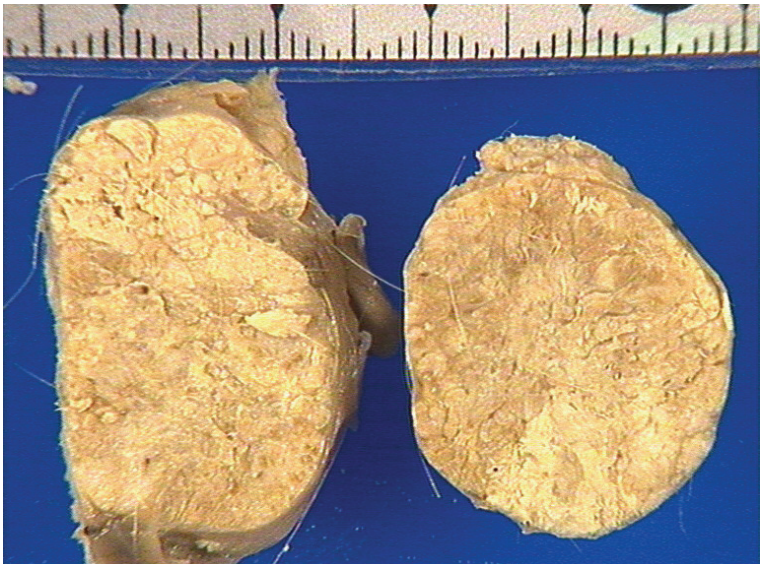

Figure 2.16: Cross-section of tumour (Rat Y3, 970-MHz PW group).

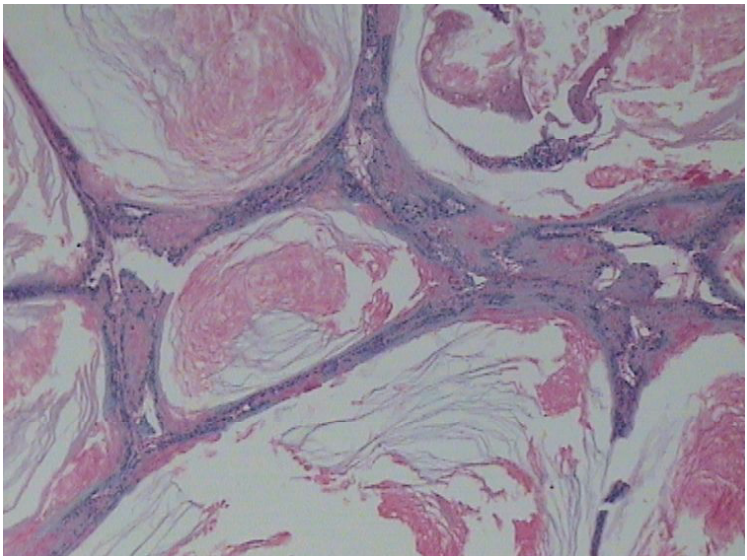

Figure 2.17: Histological preparation of tumour of rat Y3, keratoacanthoma (Rat Y3, 970-MHz PW group).

C. Rat Y4 of the 970-MHz PW group

The external and internal examination is described in Table 2.17.

External examination	
Weight	437.56 g
General aspect	Normal, 197.60 g
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.54 g
Cerebellum	0.65 g
Heart	2.66 g
Lungs	3.52 g
Liver	28.34 g
Spleen	Normal, 0.79 g

Kidneys	Minimal, irregular surface, bilateral, 3.71 g
Adrenal glands	1 missing; not weighed
Digestive tract	Normal
Stomach	Normal
Pancreas	Atrophic
Testes	At cut surface: cystic, bilateral, 4.07 g

Table 2.17: Anatomopathological data of rat Y4.

The liver presents a slight multifocal macrovesicular hepatocellular vacuolation (lipidosis). A moderate bilateral, diffuse, chronic, progressive nephropathy is observed in kidneys.

D. Rat Y10 of the 970-MHz PW group

The external and internal examination is described in Table 2.18.

External examination	
Weight	463.61 g
General aspect	Normal
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.58 g
Cerebellum	0.54 g
Heart	4.35 g
Lungs	3.23 g
Liver	Diffuse, severe, yellow discoloration, 18.35 g
Spleen	Normal, 155 g
Kidneys	Severe, irregular surface, bilateral, 5.25 g
Adrenal glands	Periadrenal cyst (0.3 x 0.3 x 0.3 cm) 0.22 g
Digestive tract	Normal

Stomach	Normal
Pancreas	Atrophic
Testes	Unilateral white foci, unilateral small, cystic, 2.54 g

Table 2.18: Anatomopathological data of rat Y10.

The ileum presents mesothelioma on the serosa. A marked, diffuse, macrovesicular, hepatocellular vacuolation (lipidosis) characterizes examined liver tissue. Mesothelioma is found. Kidneys present a severe, bilateral, diffuse, progressive nephropathy. A cystic lymph node with a metastatic mesothelioma is observed on the serosa of the adrenal glands. Testes present a unilateral Leydig cell adenoma associated with severe testicular atrophy with calcification associated with mesothelioma on the serosa. The spleen is characterized by a marked diffuse presence of foci of extramedullar haematopoiesis.

E. Rat Y14 of the 970-MHz PW group

The external and internal examination is described in Table 2.19.

External examination	
Weight	781.58 g
General aspect	Well demarcated skin nodule (10.0 x 10.0 x 7.0 cm), 197.60 g
Fur	White – yellow, clean
Internal examination	
Cerebrum	1.76 g
Cerebellum	0.76 g
Heart	3.10 g
Lungs	Multiple white pinpoint foci, all lobes, 4.26 g
Liver	28.34 g
Spleen	Normal, 1.01 g

Kidneys	Moderate, irregular surface, bilateral, 4.53 g
Adrenal glands	0.15 g
Digestive tract	Normal
Stomach	Normal
Pancreas	Atrophic
Testes	Left testis enlarged, 2.60 g (Left: 1.75 g)

Table 2.19: Anatomopathological data of rat Y14.

The macroscopically observed skin nodule consists of an infiltrating mass of pleiomorphic spindle and oval cells, with marked anisokarrhyosis, organized in whorls and interwoven bundles. This is typically a sarcoma; extra staining is necessary to further identify the type of neoplasma.

2.2. Summary

It is impossible to draw definitive conclusions from the analysis of only 19 rats, from four different exposed groups. The number is too small to make intergroup comparisons and draw conclusions on the present data.

	Sham-Exposed	9.70 GHz CW	970 MHz CW	970 MHz PW
Number of animals examined	2	5	7	5
Number of tumours	1	5	7	4

Table 2.20: Summary table of neoplastic lesions.

We can only observe that tumours have developed during the exposure periods, either benign or malign.

Further research is necessary. It is possible that some pathology occurs more or less spontaneously in older laboratory rats, like chronic progressive nephropathy and testicular atrophy. This has to be cleared out after histopathological analysis of all rats. Therefore cadavers are kept in a fixation solution till the organs can be analysed.

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Conclusion

There are three parts in this conclusion. One summarizes the results we have obtained. In the second, we present personal comments that we consider coming out of these results. In the third, we present suggestions for future research, on the basis of the results.

We have exposed four groups of 31 rats (sham-exposed, 970-MHz CW, 970-MHz PW, and 9.70-GHz CW), simultaneously, two hours a day, seven days a week during 21 months. The effect of two frequencies, together with two exposure modes, continuous wave and pulsed waves, has been investigated.

In the discussion related to the blood analysis results, we considered an exposure period of 18 months, because the last blood sampling has been performed 18 months after the start of the exposure. After this 18^{th} month, the exposure of the rats has continued for three months to prolong the total exposure period. These three more months of exposure increase the surveillance period in relation with the mortality study we performed. The results are described in Part III, Chapter 2. After those 21 months of exposure, the rats have been kept for another three months, but without exposure.

Because of practical constraints related to blood sampling and feasibility of blood analyses, the 18 first months of exposure have been subdivided in five periods of approximately three months. In practice, blood samplings have been performed after 0, 3, 8, 11, 14, and 18 months of exposure.

After each period, we have compared the parameters of the exposed groups with those of the sham-exposed group. In part II of this thesis the results of the blood analysis have been presented. Several statistically significant

differences have been found between each of the exposed groups and the sham-exposed group.

Synoptic tables in chapter 1 of Part II summarize the parameters for which a statistically significant result has been obtained in at least one out of the three exposed groups for each period. The statistically significant values ($p < 0.05$) have been marked with an asterisk in those synoptic tables. For the sake of completeness, the values for the other exposed groups - even if not significant - are filled out in the table too.

The most obvious result is the findings in the monocytes, where a remarkable increase is brought to the fore after both a 3-month and 8-month exposure to microwaves.

Monocytes are part of the immune system and are the biggest of all leucocytes. Monocytes are the precursors of macrophages which play a role in the elimination of bacteria, fungi, particles foreign to the body and dead or damaged cells.

It is very remarkable that in all the exposed groups, one can notice an increase compared to the sham-exposed group. This finding points to a stress response in the blood-forming system after a long-term exposure to low-level microwaves. A possible hypothesis is that the exposure induces an increased myelopoietic reaction which results in a stimulation of the monocyte formation in the blood. It is as if the living organism reacts to a foreign aggression or intrusion.

The statistical significant increase in erythrocyte count and changes in derived indices after three months of exposure is another indication supporting the hypothesis of a stimulating effect of microwave exposure onto the haematopoiesis.

After 11 and 18 months of exposure, statistically significant increases in other types of leukocytes are demonstrated. Leucocytes and neutrophils show an increase of about 30% compared to the sham-exposed group after 18 months of exposure. This may be an indication of a long-term effect, even under low-thermal conditions.

When the entire exposure period is considered, statistically significant results are observed in the CW groups as well as in the PW group. The number of statistically significant results found in the PW group is about the half of the number in the CW groups (8 *vs.* 15).

As behaviour is the most sensitive measure of biological effects, we evaluated the feasibility of setting-up a behavioural study with rats that have been exposed to microwaves. We put two populations to the test. Each population was composed of an effective exposed group of rats and a sham-exposed group. One population was the 970-MHz PW exposed group that already has been exposed for 15 months. The second population was a group of rats that has been exposed for two months. Both groups were subject to an object recognition task. We found that the rats that have been exposed for two months show normal exploratory behaviour. The rats that have been exposed for 15 months do not make the distinction between a familiar object and an unknown object. It has to be said that this experiment was set-up only as a feasibility study for further research related to behavioural changes due long-term microwave exposure and therefore we used the group of rats that already has been exposed for 15 months in another experiment. However, this was the case for the related sham-exposed group either. Our finding has to be considered more as a trend than as an undisputable result, but the trend is obvious.

On its turn, the mortality study which has been described in Part III, Chapter 2, points towards a biological effect of microwaves. All the exposed

groups show survival rates beneath the mean survival rate of non treated rats of the same age.

This effect becomes clearer after a three-month follow-up period after the exposure has been stopped. Roughly, the mortality rate in the exposed groups is twice that of in the sham-exposed group. An increased mortality can be consistent with an alteration in the monocyte count and/or total leucocyte count in the peripheral blood, since leucocytes take part in an overall immune response of the body against foreign organisms in the broadest sense.

We continue by giving some personal comments. First of all, an important reminder in these is that one must be careful when wishing to extrapolate results obtained on animals to possible effects on human beings. This requires an understanding and appreciation of biophysical principles, interspecies scaling, and the selection of biomedical parameters that reflect basic physiological functions. Because of the use of animals as a surrogate for humans in hazard analysis, one must create a set of experimental conditions which are as relevant as possible for the purpose of the study. Many factors, such as methods of animal care, the role of circadian rhythms, temperature and humidity, etc., as well as physiological interactions, must be considered in experimental design and analysis of the results. One should not extrapolate to man, results obtained in small laboratory animals without consideration of size. To produce an identical whole-body average specific absorption rate one must scale from one frequency to another. We may state that we have taken into account as much as practically possible these advices in the design of the experimental protocol.

1. Our study was based on low-level long-term exposure. Long-term here is defined as an exposure for a very significant part of rat lifetime. One can

roughly state - by way of an indication - that 21 months of rat exposure corresponds to a human exposure during 63 years.

2. Our results give one more indication that there are microwave biological effects. As there exist vulnerable subpopulations in our society (people with a weak immune system, children, elderly,…) the application of the precautionary principle should be advisable.

3. As already mentioned in literature, we observed that trends on behavioural effects can be observed after a shorter exposure time than physiological results can. This is certainly true when the entire exposure period annex follow-up period is considered.

4. Increased mortality of the exposed rats with respect to non-exposed rats is a significant result. This tendency remains when we compare this mortality rate with mortality in a reference group from the breeding colony where our rats originated from. The mortality rate of the sham-exposed group is similar to the mortality rate in this reference group, while the mortality rate in exposed groups is nearly 50% higher than in this unexposed reference group.

Very interesting to observe is the fact that while mortality is significantly higher at the age of 25 months (including a 21-month exposure), mortality increases to about twice mortality of non-exposed rats after a further 3-month non-exposure period of time.

A clear reason for the increased mortality in microwave exposed groups has not been identified yet. It may be due to a weakening of the immune system, favouring the genesis of decay processes which may result in life-threatening disorders including cardiovascular and pulmonary diseases, cancer or premature aging. The anatomopathological analysis of all rats may assist in finding some more explanations for the increased mortality.

This leads us seamlessly to the last part of the conclusions, namely the recommendations for further research. We should like to introduce them by the following preamble. In its first recommendation (Reference 5, 1993, Chapter 2, Part I), WHO mentions: *In normal thermal environments an SAR of 1-4 W/kg for 30 minutes produces average body temperature increases of less than 1°C for healthy adults*. It then states that this is not to be considered as harmful for human being. On may agree with this, noting carefully, however, that it goes about *a 30-minute exposure of healthy adults*. It should also be well noted that WHO recommends a safety factor of 50, starting however from 4 W/kg while the temperature it wants to limit may be observed for *an SAR of 1-4 W/kg*. This brings the value not to be exceeded at 0.08 W/kg.

In its well-known paper (Reference 10, 1998, Chapter 2, Part I), ICNIRP maintains the same recommendation and the same safety factor, also calculated from 4 W/kg, extending it however to *permanent exposure and to all human beings*, while it does not explicitly justify those two significant extensions.

This ambiguity is the reason why we have led our animal study to bring a contribution based on an exposure at low level, calculated for rats the way WHO and ICNIRP have established it for human beings with the principal length as the only differentiating parameter, and for long-term, equal to about 75% of the rat lifetime.

The results we have obtained are opening some doors for future research. The subject of this doctoral thesis is topical and the scientific interest is obvious.

The cadavers of the 124 rats are available for anatomopathological investigation. This will be quite an amount of work. We have analysed a limited number of bodies. Futher thorough analysis will be necessary, for

instance to check if small tumours can be detected, possibly in specific organs. This is related to research on possible causes of illness that produced premature mortality.

Theoretical research, combined with anatomopathological investigation, should answer the question whether microwave exposure can possibly have been the cause of *premature aging* of the rats. Effects on the immune system, on the nervous system, and on the olfactive bulb should certainly be investigated. A hint is that direct microwave effects occur only in the first skin depths, mainly the first one, although this represents a larger part of the body in the rat than in human being.

New experimental low-level long-term exposure studies should be led. This is also the strong conviction of the professors, scientists, and academic people working in this field which whom I had very fruitful discussions and who surrounded me during my doctoral study. In particular, since most if not all of the new devices and systems are based on pulse-amplitude modulation, comparison should be made between a 970-MHz-CW exposure and an actual 970-MHz-GSM transmitter. Research should indeed investigate whether pulse-amplitude modulated microwaves (*e.g.* radars) are able to produce biological effects different from analogue-modulated microwaves. An effort should be necessary, certainly about the possible direct demodulation by a living organism of the extremely-low-frequency components contained in the baseband signal. It still remains an open question whether non-constant power variation with time has any significant biological effect.

Furthermore, experimental low-level long-term exposure studies should also be made between about a 10-GHz-CW exposure and a 10-GHz-PW exposure. This however requires a high-power 10-GHz transmitter or an amplifier. Especially the introduction of a 9.70-GHz PW group in a new

protocol is highly advisable, because of the omnipresence of new technologies, both in military as in civilian applications. Most, if not all of those new devices are based on pulse-amplitude modulation of the carrier wave and are working at more and more higher frequencies. It is therefore essential to be aware of the rapid development of new sources of non-ionizing radiation – both in professional and home environment – and to continue the scientific assessment on the various health aspects related to these innovations. This would include different exposure scenarios with regard to simultaneous exposure to complex multiple frequencies spread over a potentially large frequency range.

Besides, as the 9.70-GHz CW amplifier broke down during the ongoing of our experiment, it is recommended to introduce the 9.70-GHz CW exposure in the new protocol enabling a longer exposure period than it was the case in the present study. Only under these conditions, the possible different biological effect emanating from pulsed waves can be compared to long-term continuous wave exposure.

Appendix A: Statistical Analysis of Period 2 (P2)

After 8 months of exposure (2 hours/day, 7 days/week) a second blood sampling is performed. Similar to the first period, the results of the analysis were submitted to an ANOVA test for multiple comparisons.

The ANOVA test showed a significant difference between the means of the following parameters: monocytes, erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC). A Dunnett test is used as post-hoc to correct for multiple comparisons.

A.1. Monocytes P2

Similar to the first 3-months period of exposure, an increased expression of monocytes is detected in all the three real exposed groups in comparison with the sham-exposed group. This can be seen in Figure A.1. The most obvious change attributable to the microwave exposure was the sharp increase (20.6%) in the monocyte count of the 970-MHz CW group (M = 3.334, SD = 0.9075) compared to the sham-exposed group (M = 2.765, SD = 0.837). Another meaningful increase was found in the 9.70-GHz CW group (M = 3.237, SD = 0.736) where the monocyte count was 17.1% higher than in the sham-exposed group. The increase in the 970-MHz PW group (M = 2.997, SD = 0.799) was about 8.4%. These findings have to be tested on their statistical significance with a Dunnett test.

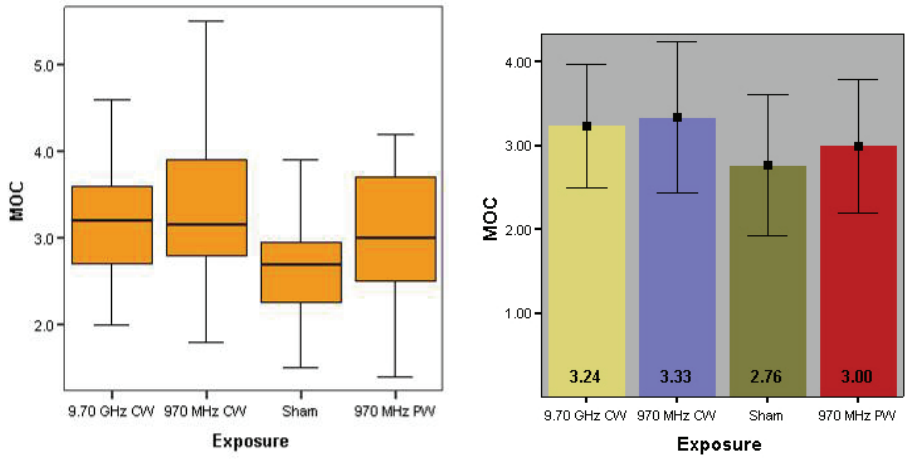

Figure A.1: Descriptive analysis of monocyte count in the four groups after 8-month exposure.

With p = 0.630, the Levene test is not significant at the 0.05 level, indicating that the sample variances are equal. Therefore, the null hypothesis H0, stating that there is no difference in monocytes between the exposed group and the sham-exposed group, can not be rejected.

Before, the normality of the distribution has been verified and confirmed.

The Dunnett test compared the mean value for the monocyte count of the exposed rats with those of the sham-exposed rats. This comparison was found to be statistically significant for the 970-MHz CW exposed rats (p = 0.019). This result indicates with a risk of error of 1.9% that the 970-MHz CW exposed rats were evaluated as showing a higher (20.6 %) monocyte count than the rats of the sham-exposed group. The mean difference of 0.472 (3.237 – 2.765) between the sham-exposed group and the 9.70-GHz CW group, an increase of 17.1%, was not found to be statistically significant (p = 0.070).

Comparing the 970-MHz PW and the sham-exposed group learns that the increase in the exposed group is about 8.4%. The Dunnett test couldn't elucidate this difference was still statistically different at a 0.05 level ($p = 0.557$).

A.2. Haemoglobin P2

The haemoglobin concentration, expressed in g/100ml, is retained for further analysis. The box plot representation related to the first 8 months of exposure shows the descriptive statistics related to the haemoglobin concentration. This is depicted in Figure A.2.

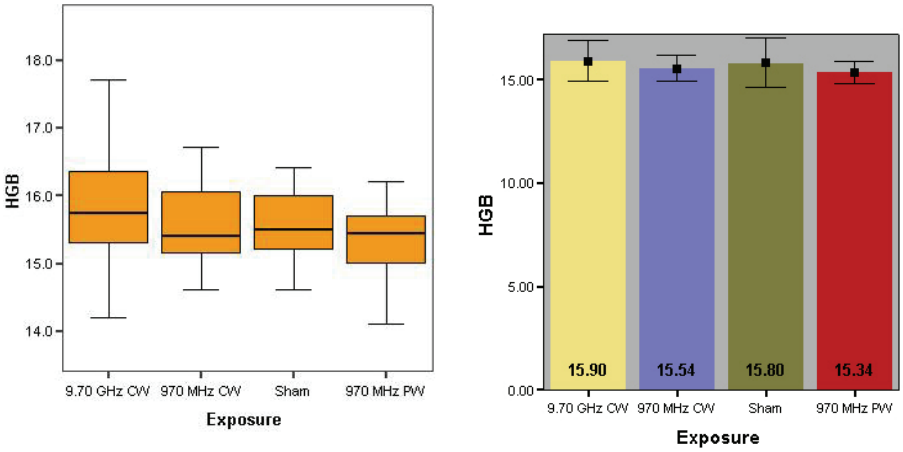

Figure A.2: Descriptive analysis of haemoglobin concentration in the four groups after 8-month exposure.

In stead of the findings after the first three months of exposure, only a marginally increase (0.6%) is found between the 9.70-GHz CW group ($M = 15.9000$, $SD = 0.9699$) and the sham-exposed group ($M = 15.8030$, $SD = 1.1734$). The 970-MHz CW ($M = 15.5390$, $SD = 0.6312$) group and the 970-MHz PW group ($M = 15.3400$, $SD = 0.5250$) show another

tendency: a decrease compared to the sham-exposed group of 1.7 % and 2.9%, respectively. After performing a Dunnet T3 test (Levene's test with $p = 0.018$) none of these differences are found to be statistically significant ($p > 0.05$). The p-values are $p = 0.858$, $p = 1.000$, and $p = 0.295$ for the 970-MHz CW, the 9.70-GHz CW, and 970-MHz PW exposure, respectively.

A.3. MCHC P2

Figure A.3 shows the MCMC parameter, expressed in g/dl, in the four groups of rats after a period of eight months of continuously exposure.

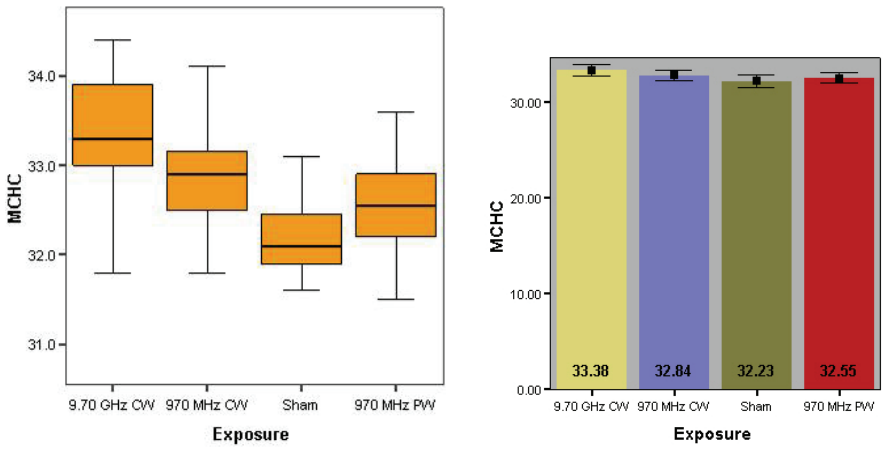

Figure A.3: Descriptive analysis of mean corpuscular haemoglobin concentration in the four groups of rats after 8-month exposure.

One can observe a higher level of this variable in all exposed groups in comparison with the sham-exposed. The increase is the most pronounced in the 9.70-GHz CW group.

This finding has to be tested on statistical significance using a Dunnett test.

A Dunnett test can be applied, because, according to the Levene test ($p = 0.720$) the variances are equal in all samples; the normality of all

distributions has been verified and confirmed by a Kolmogorov-Smirnov test.

The mean difference of 1.148, which represents a 3.6% increase between the sham-exposed group (M = 32.2290, SD = 0.6654) and the 9.70-GHz CW group (M = 33.3770, SD = 0.6558), was found to be statistically significant (p = 2.35 10-8).

At the same time, the increased level of MCHC in the 970-MHz CW group (M = 32.8440, SD = 0.5224) is compared to the MCHC level in the sham-exposed group. The level of MCHC in the 970-MHz CW group is 1.9% higher and differs in this significantly from the sham-exposed group (p = 2.53 10-4).

The 970-MHz PW group shows a slightly increase of 1.0%. The comparison between the 970-MHz PW (M = 32.5470, SD = 0.5450) and the sham-exposed group, learns that there is no statistically significant difference (p = 0.104).

A.4. MCV P2

Since we observed in the previous analysis statistically significant differences in haemoglobin related variables between exposed groups and the sham-exposed group, it should be logical to put the mean corpuscular (cell) volume (MCV) to the test; it is expressed in femtoliter (fl, 10-15 l).

Figure A.4 displays the levels in the four groups.

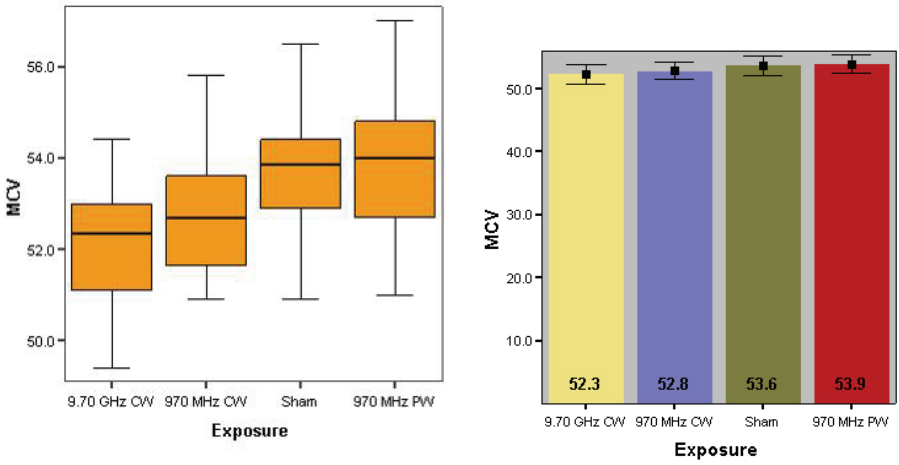

Figure A.4: Mean corpuscular volume (fl) after 8 months of exposure in the 4 groups of rats.

The 970-MHz PW group shows a 0.4% increase compared to the sham-exposed group, the 970-MHz CW has a 1.5% lower MCV level than the sham-exposed group; the 9.70-GHz CW group exhibits a 2.5% lower level than the sham-exposed group.

Only the rats of the 9.70-GHz CW group (M = 52.317, SD = 1.5702) differ significantly from the MCV value in the sham-exposed rats (M = 53.640, SD = 1.5467) according to the Dunnett test (p = 0.002). The decrease in the 970-MHz CW group is not statistically significant (p = 0.075), nor is the small increase in the 970-MHz PW compared to the sham-exposed group (p = 0.882).

A.5. Reticulocytes P2

All the exposed groups present higher values than the sham-exposed group. This can be seen in Figure A.5.

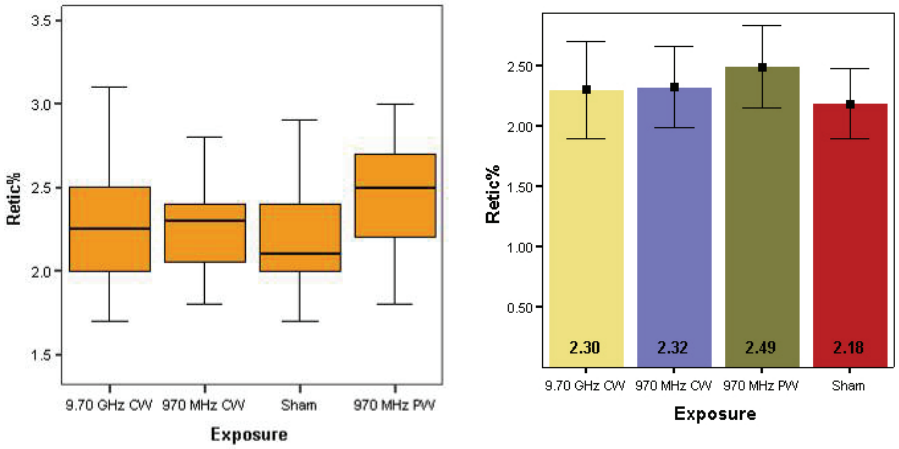

Figure A.5: Reticulocyte count in the 4 groups of rats after 8 months of exposure.

The reticulocyte count in the 970-MHz PW group (M = 2.490, SD = 0.3377) is 14.01% higher than in the sham-exposed group (M = 2.184, SD = 0.2945).

The reticulocyte count in the 9.70-GHz CW group (M = 2.300, SD = 0.4034) is 5.31% higher than in the sham-exposed group.

The mean value in the 970-MHz CW group (M = 2.319, SD = 0.3390), is 6.18% higher than the mean value in the sham-exposed group.

The homogeneity of variances and the normality of the distribution have been tested and have been confirmed before performing the Dunnett test.

Only the difference between the 970-MHz PW group and the sham-exposed group is statistically significant (p = 0.002).

A.6. Erythrocytes (RBC) P2

The results of the erythrocyte or red blood cell count after a period of eight months continuously exposure during two hours a day, are graphically represented in Figure A.6. The figures have to be multiplied by 10^6 and stand

for the number of erythrocytes per µl blood. As shows Figure 1.5. (Part II. Chapter 1), the situation is totally different to the one in the first exposure period, *i.e.* after 3 months of exposure, where all the exposed groups had a higher level of RBC than the sham-exposed group.

In contrast, after eight months of exposure, all the groups demonstrate a lower red blood cell count than the sham-exposed group, except the 9.70-GHz CW group, which value is only 0.4% higher. Unlike the former exposure period, the 970-MHz PW group ($M = 8.7603$, $SD = 0.36652$) differs to a larger extent (4.0%) in relation to the sham-exposed group ($M = 9.1272$, $SD = 0.69034$), followed by the 970-MHz CW group ($M = 8.9635$, $SD = 0.44051$), with a RBC count which is 1.8% lower than the sham-exposed group.

The number of rats in each group (30 rats in the 970-MHz PW group, 29 rats in the sham-exposed group) is sufficiently high to assume on a correct basis that the variable follows a normal distribution in both populations. This has been confirmed by a Kolmogorov-Smirnov test.

The variances in all samples are not equal which is explained by the Levene test ($p = 0.005$); therefore a Dunnett T3 test was performed.

The Dunnett T3 test does not reveal any statistically significant difference at a 0.05 significance level. The p-values are 0.085, 0.854 and 1.00 for 970 MHz PW, 970 MHz CW and 9.70 GHz PW, respectively.

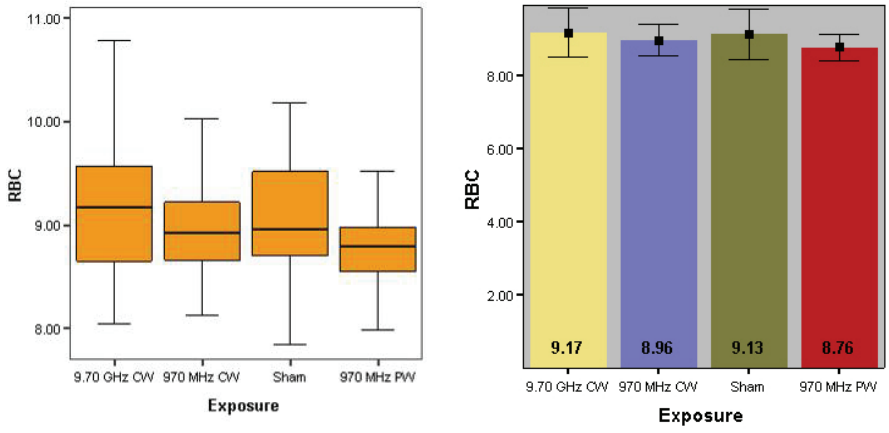

Figure A.6: Red blood cell count after 8 months of continuous exposure in the 4 groups of rats.

A.7. Leucocytes (WBC) P2

The leucocyte count is expressed in 10^3 cells per µl. Figure A.7 shows the white blood cell count after the first period of an 8-month continuous exposure period at a rate of two hours per day.

Unlike the former period of three months of exposure, no statistically significant difference between exposed groups and sham-exposed group was found on the basis of a Dunnett T3 test (Levene's test with $p < 0.05$).

The white blood cell count in the 970-MHz CW group ($M = 5.539$, $SD = 0.8131$) is 2.6% higher ($p = 0.999$) than in the sham-exposed group ($M = 5.4000$, $SD = 1.7288$), whereas the white blood cell count in the other groups is lower than in the sham-exposed group: the 970-MHz PW ($M = 5.024$, $SD = 0.9080$) displays a 7.0% lower WBC count ($p = 0.863$); the 9.70-GHz CW group ($M = 5.3370$, $SD = 0.6955$) a 1.2% lower value ($p = 1.000$).

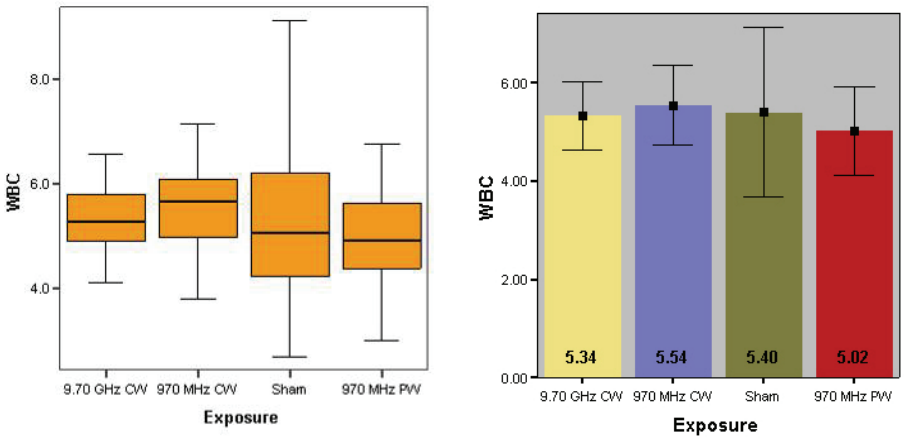

Figure A.7: White blood cell count related to the four groups of rats, after 8 months of exposure.

A.8. Eosinophils P2

There is a main difference between the sham-exposed group and the real exposed groups in a sense that the eosinophils value is the most elevated for the non-exposed rats (Figure A.8).

This is a similar finding as in the former period, *i.e.* after the first three months of exposure. The eosinophil count in the peripheral blood of the 970-MHz PW (M = 1.770, SD = 0.6342) is 12.6 % lower than in the sham-exposed group (M = 2.026, SD = 0.6207) after eight months of exposure; the 9.70-GHz CW group (M = 1.8600, SD = 0.5347) shows a decrease of 8.2% compared to the sham-exposed rats, and the 970-MHz CW group (M = 1.9320, SD = 0.5884) a decrease of 4.6%. These findings have to be tested on their statistical significance with a Dunnett test. The homogenity of the variances is verified and conformed by Levene's test (p = 0.906).

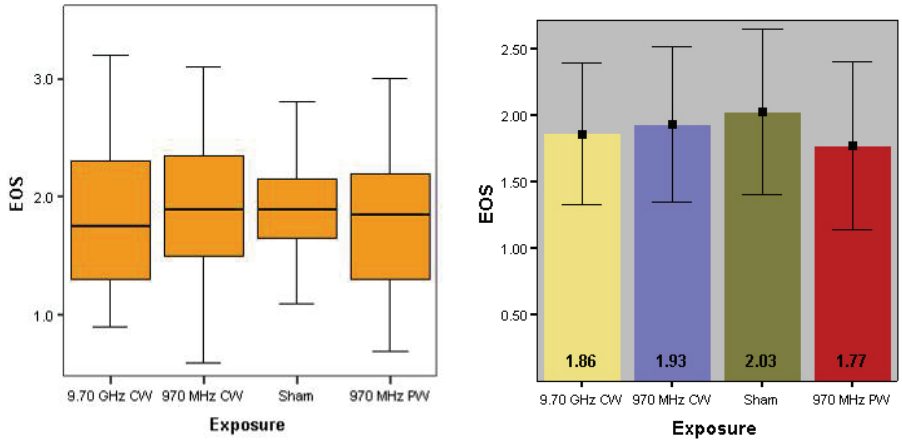

Figure A.8: Eosinophils for different exposure types after an 8-month exposure period.

Unlike the former considered period of 3 months of exposure, the results of the statistical analyses indicate that the three types of exposure were unable to induce a significant variation related to the eosinophil parameter after eight months of exposure ($p > 0.05$).

A.9. ACTH P2

The descriptive statistical analysis graphically depicted in Figure A.9, displays the ACTH (pg/ml) in the peripheral blood of the four groups of rats after eight months of exposure. A decreased expression is detected in all the exposed groups compared to the sham-exposed group.

Like in the former exposure period, after the first three months, the highest difference in ACTH concentration (12.0% decrease) is found between the sham-exposed group ($M = 196.0800$, $SD = 114.2098$) and the 970-MHz PW exposed group ($M = 172.6020$, $SD = 68.6802$).

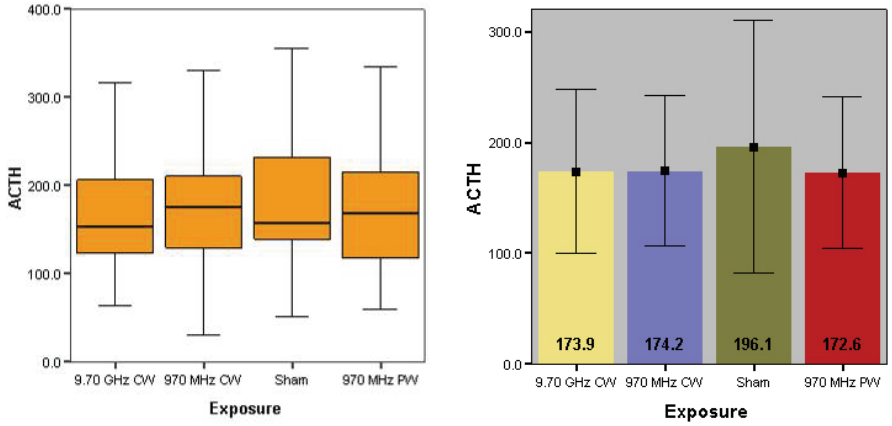

Figure A.9: Descriptive analysis of ACTH (pg/ml) in the four groups after 8 month of exposure.

The difference between the 9.70-GHz CW group (M = 173.8750, SD = 74.2724) and the sham-exposed group is of the order of 11.3%, while the decrease in the 970-MHz CW group (M = 174.1880, SD = 68.0222) is about 11.2%.

It is worthwhile to investigate these findings on their statistical significance, using a Dunnett test with a level of significance of $p < 0.05$ (Levene's test for homogenity with $p = 0.266$).

In none of the cases, a statistically significant effect of the exposure could be detected ($p > 0.05$).

A.10. Synopsis

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 2.6%	- 7.0%	- 1.2%
Monocytes	+ 20.6%*	+ 8.4%	+ 17.1%**
Erythrocytes (RBC)	- 1.8%	- 4.0%**	+ 0.4%
Haemoglobin	- 1.7%	- 2.9%	+ 0.6%
MCV	- 1.5%**	+ 0.4%	- 2.5%*
MCHC	+ 1.9%*	+ 1.0%**	+ 3.6%*
ACTH	- 11.2%	- 12.0%	- 11.3%
Reticulocytes	+ 6.18%	+ 14,01%*	+ 5.31%
Eosinophils	- 4.6%	- 12.6%	- 8.2%

Table A.1: Parameters presenting a statistically significant difference according to Dunnett (*) or only Student (**), compared to the sham-exposed group, after 8 months of exposure.

Appendix B: Statistical Analysis of Period 3 (P3)

In this chapter we analyze the blood parameters of 11-months-exposed rats on their statistical significance in relation to the sham-exposed rats.

The ANOVA shows significant differences in the means of the following parameters: haematocrit, mean corpuscular haemoglobin concentration and reticulocytes. Since each distribution is homogene according to Levene's test ($p > 0.05$), a Dunnett test can be performed to evaluate if these differences are robust against multiple comparisons.

B.1. Monocytes P3

After 11 months of exposure, we still observe an increase in monocytes in all the exposed groups compared to the sham-exposed group (Figure B.1). This is a similar tendency as in the former exposure periods. The rise in the 970-MHz CW group is the most obvious (13%), followed by the 9.70-GHz CW group (9.6%). The relative increase concerning the 970-MHz PW group is 8.8%.

Before performing the Dunnett test the normality of the distribution and the equality of the variances have been verified and confirmed. With $p = 0.822$, The Levene test is not significant at the 0.05 level, indicating that the sample variances are equal.

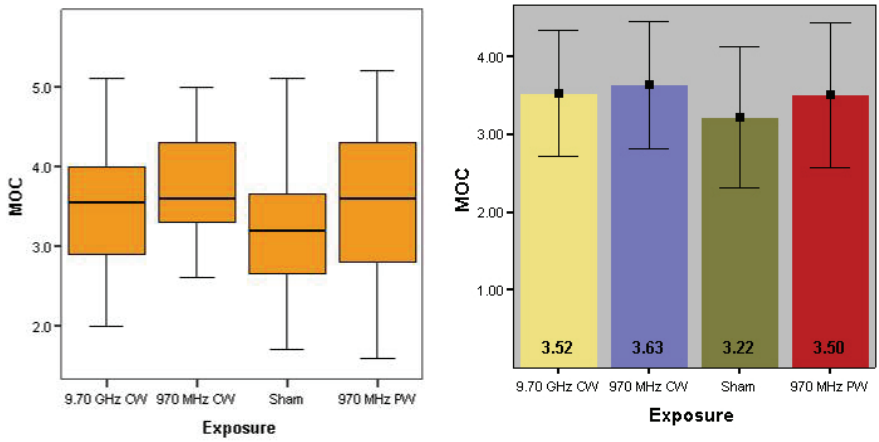

Figure B.1: Descriptive analysis of monocyte count for different exposure types after an 11-month exposure period.

Using the Dunnett test, we compared the mean value of the monocyte count of the 970-MHz CW exposed rats (M = 3.634, SD = 0.8169) with this of the sham-exposed rats (M = 3.216, SD = 0.9023). The mean difference of 0.418 between the sham-exposed group and the 970-MHz CW group, was found to be statistically insignificant (p = 0.160).

The mean difference of 0.307 between the sham-exposed group and the 9.70-GHz CW group (M = 3.523, SD = 0.8118), was not statistically significant (p = 0.379).

Comparing the 970-MHz PW (M = 3.500, SD = 0.9381) and the sham-exposed group learns that the increase in the exposed group is about 8.8 %. The Dunnett test couldn't elucidate this difference was still statistically different (p = 0.450).

B.2. Haemoglobin P3

The box plot representation related to the first 11 months of exposure shows the descriptive statistics related to the haemoglobin concentration (g/dl). This is depicted in Figure B.2.

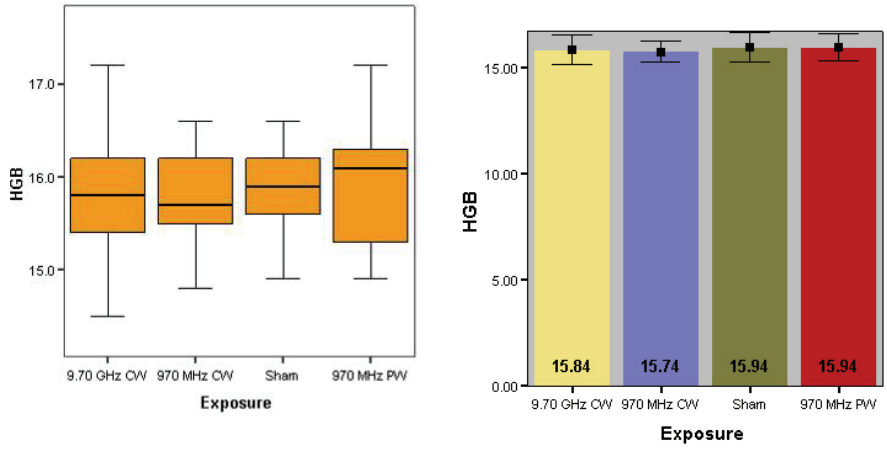

Figure B.2: Descriptive analysis of haemoglobin concentration in the four groups after 11-month exposure.

Like in the former exposure period of 8 months, only a marginally increase (nearly 1%) is found between the exposed groups and the sham-exposed group. The mean haemoglobin concentration in the 970-MHz CW group (M = 15.743, SD = 0.4876) is 1.3 % decreased in comparison with the sham-exposed group (M = 15.942, SD = 0.7061). The haemoglobin concentration in the 9.70-GHz CW group (M = 15.841, SD = 0.7020) is 0.6% decreased in comparison with the sham-exposed group.

The 970-MHz PW group (M = 15.941, SD = 0.6339) has similar values as the sham-exposed group.

None of these results are statistically significant at $p < 0.05$.

B.3. MCHC P3

Figure B.3 shows the MCMC parameter, expressed in g/dl, in the four groups of rats after a period of eleven months of continuously exposure. One can observe a higher level of this variable in all exposed groups in comparison with the sham-exposed. The increase is in the order of 3%. This finding has to be tested on statistical significance using a Dunnett test with a level of significance of $p < 0.05$.

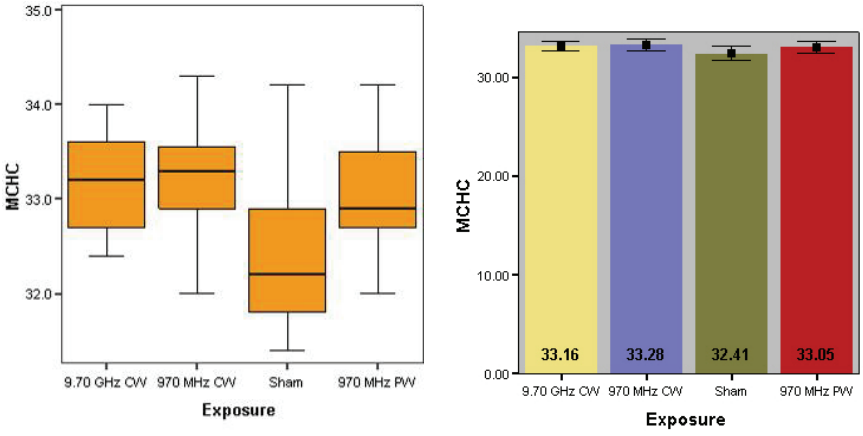

Figure B.3: Descriptive analysis of mean corpuscular haemoglobin concentration in the four groups of rats after 11-month exposure.

According to the Levene test for equality of variances the sample variances are equal in both of the two distributions which have been compared ($p > 0.05$).

The normality of the distribution has been verified and confirmed.

The mean difference of 0.867 between the sham-exposed group ($M = 32.4100$, $SD = 0.7236$) and the 970-MHz CW group (33.2770, $SD = 0.5690$) was found to be highly statistically significant ($p = 4.12\ 10^{-7}$).

This difference corresponds with a 2.7% increase in MCHC value comparing to the sham-exposed group.

The value of MCHC in the 9.70-GHz CW group (M = 33.1600, SD = 0.4724) is 2.3% higher and differs in this significantly ($p = 1.26\ 10^{-5}$) from the sham-exposed group (M = 32.4100, SD = 0.7236).

Comparing the 970-MHz PW group (M = 33.0520, SD = 0.6328) and the sham-exposed group learns that there is also a statistically significant difference concerning MCHC ($p = 2.30\ 10^{-4}$). The 970-MHz PW group shows an increase of 2.0%.

B.4. MCV P3

Since we observed in the previous analysis statistically significant differences in haemoglobin related variables between exposed groups and the sham-exposed group, it should be logical to put the mean corpuscular (cell) volume to the test; it is expressed in femtoliter (fl, 10^{-15}l).

Only the rats exposed to 970-MHz CW (M = 53.6160, SD = 1.1495) differ significantly (p = 0.030) from the sham-exposed group (M = 54.5800, SD = 1.5610); the 9.70-GHz CW group (M = 53.7930, SD = 1.6090) and the 970-MHz PW group (M = 53.9590, SD = 1.4884) do not differ significantly from the MCV value in the sham-exposed rats according to a Dunnett test with p = 0.099 and p = 0.246, respectively.

Figure B.4 displays the levels in the four groups. The 970-MHz CW group shows a 1.8% decrease compared to the sham-exposed group. The 9.70-GHz CW has a 1.4% lower MCV value than the sham-exposed group whereas the 970-MHz PW group exhibits a 1.1% lower level than the sham-exposed group.

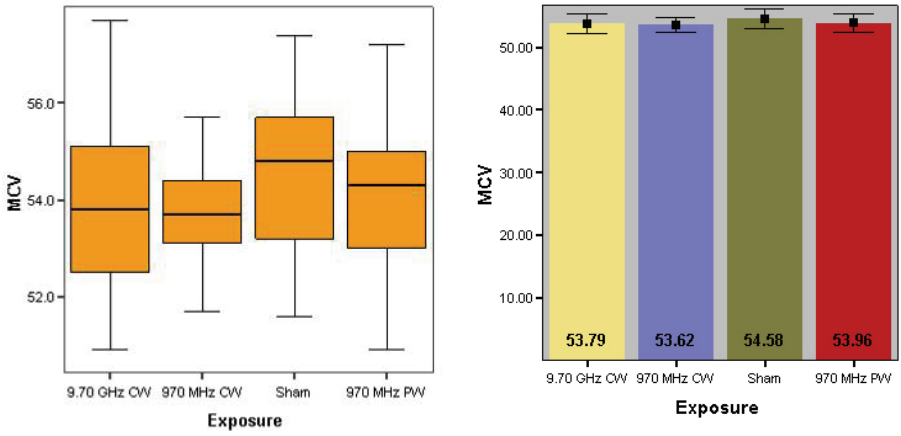

Figure B.4: Mean corpuscular volume (fl) after 11 months of exposure in the 4 groups of rats.

B.5. Reticulocytes P3

All the exposed groups present lower values than the sham-exposed group.

The homogeneity of variances and the normality of the distribution have been tested and have been confirmed before performing a Dunnett test.

The reticulocyte count (x 10^3/µl) in the 9.70-GHz CW group (M = 182.21, SD = 28.238) is 12.9% higher than in the sham-exposed group (M = 209.19, SD = 31.884). The mean difference between those two groups is statistically significant (p = 0.002).

The mean value in the 970-MHz CW group (M = 203.99, SD = 28.376) is 2.5% lower than the mean value in the sham-exposed group and is not statistically different (p = 0.839).

The reticulocyte count in the 970-MHz PW group (M = 207.44, SD = 31.060) is 0.8% lower than in the sham-exposed group. The difference is not statistically significant (p = 0.992).

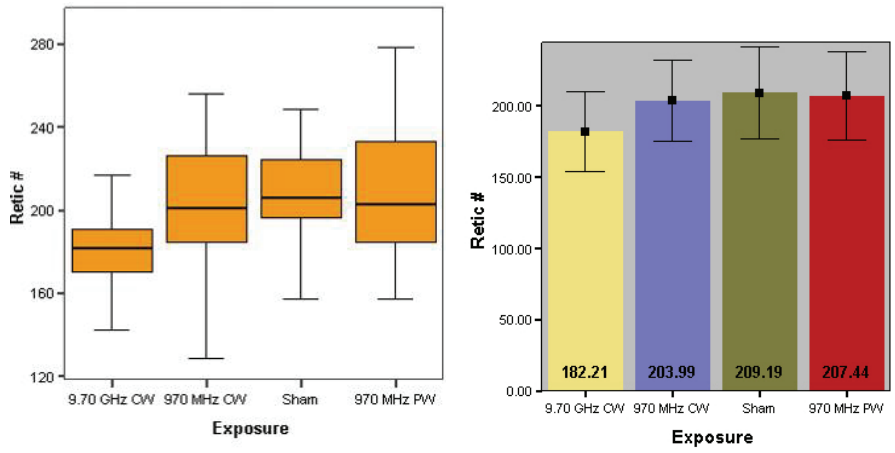

Figure B.5: Reticulocyte count in the 4 groups of rats after 11 months of exposure.

B.6. Erythrocytes (RBC) P3

The results of the red blood cell count after a period of eleven months continuously exposure during two hours a day, are graphically represented in Figure 2.6. The figures have to be multiplied by 10^6 and stand for the number of erythrocytes per µl blood.

As shows Figure B.6, the situation is similar to the former exposure period, *i.e.* after 8 months of exposure, where all the exposed groups have a lower level of RBC than the sham-exposed group.

The RBC count in the sham-exposed group (M = 8.9467, SD = 0.4204) is higher than in the 970-MHz PW group (M = 8.9417, SD = 0.3839) which is higher than the RBC count in the 9.70-GHz CW group (M = 8.8928, SD = 0.4796) and the 970-MHz CW group (M = 8.8327, SD = 0.3010). Though, the difference between the groups is negligible (about 1% difference). No statistical differences between the exposed groups and the sham-exposed groups were observed at $p < 0.05$.

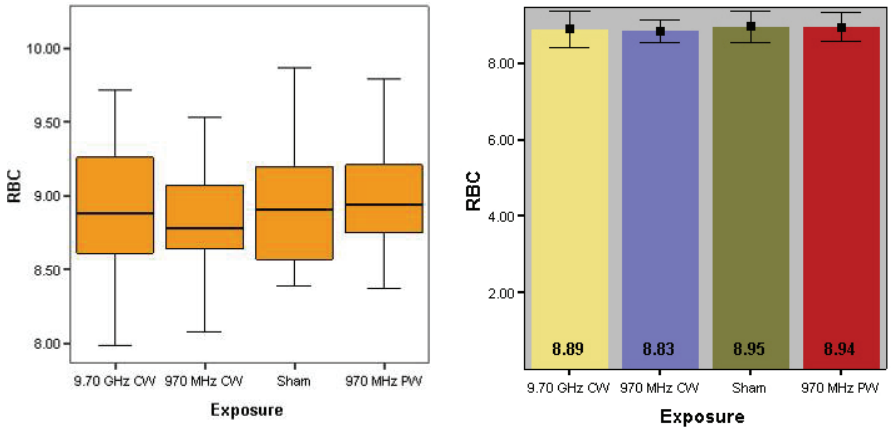

Figure B.6: Red blood cell count after 11 months of continuous exposure in the 4 groups of rats.

B.7. Leucocytes (WBC) P3

The leucocyte count is expressed in 10^3 cells per µl. Figure B.7 shows the WBC after an 11-month continuous exposure at a rate of two hours per day.

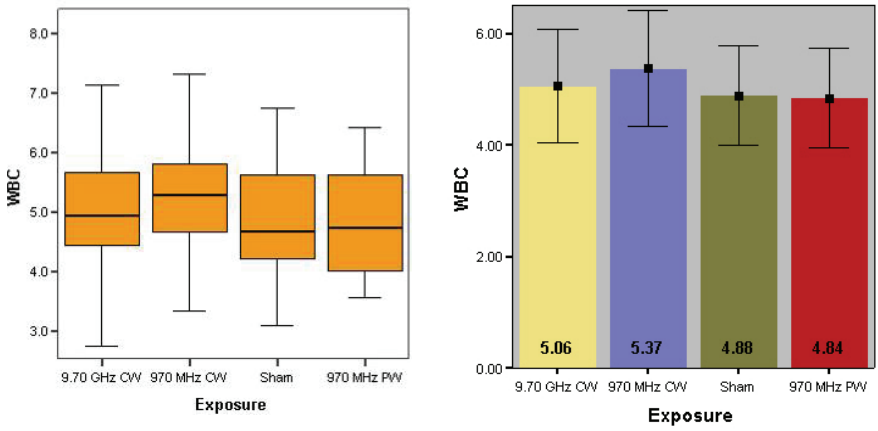

Figure B.7: White blood cell count related to the four groups of rats, after 11 months of exposure.

Similar to the former period of eight months of exposure, no statistically significant difference between exposed groups and sham-exposed group was found based on a Dunnett test.

The white blood cell count in the 970-MHz CW group (M = 5.3700, SD = 1.0426) is 9.9% higher (p = 0.131) than in the sham-exposed group (M = 4.8840, SD = 0.8957), while the white blood cell count in the other groups is lower than in the sham-exposed group: the 9.70-GHz CW (M = 5.0600, SD = 1.0288) displays a 3.6% higher WBC count (p = 0.830); the 970-MHz PW group (M = 4.8410, SD = 0.8924) a 0.9% lower value (p = 0.997).

B.8. Eosinophils P3

There is a main difference between the sham-exposed group and the real exposed groups in a sense that the eosinophils value is the most elevated for the non-exposed rats (Figure B.8).

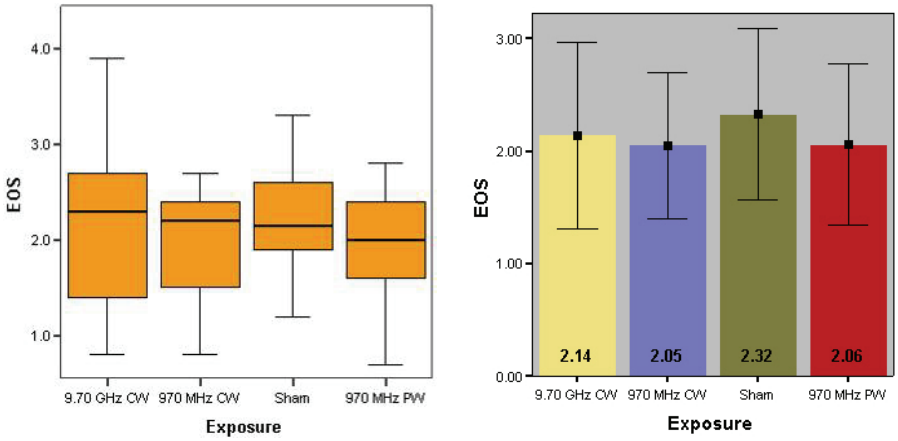

Figure B.8: Descriptive analysis of eosinophils for different exposure types after an 11-month exposure period.

This is a similar finding as in the former period, *i.e.* after eight months of exposure.

The eosinophil count in the peripheral blood of the 970-MHz CW ($M = 2.0470$, $SD = 0.6485$) is 11.9 % lower than in the sham-exposed group ($M = 2.3230$, $SD = 0.7614$) after eleven months of exposure; the 970-MHz PW group ($M = 2.0550$, $S = 0.7169$) shows a decrease of 11.5% compared to the sham-exposed rats, and the 9.70-GHz CW group ($M = 2.1370$, $SD = 0.8257$) a decrease of 8.0%. These findings have to be tested on their statistical significance with a Dunnett test. Similar to the former considered period of 8 months of exposure, the results of the statistical analyses indicate that the three types of exposure were unable to induce a significant variation ($p < 0.05$) related to the eosinophil parameter after eleven months of exposure.

B.9. ACTH P3

The descriptive statistical analysis graphically depicted in Figure B.9 displays the ACTH (pg/ml) concentration in the peripheral blood of the four groups of rats after eleven months of exposure. An increased expression is detected in all the exposed groups compared to the sham-exposed group. Like in the former exposure period, after eight months, the highest difference (28.4% increase) in ACTH level is found between the sham-exposed group ($M = 174.3626$, $SD = 70.4244$) and the 970-MHz PW exposed group ($M = 223.7936$, $SD = 96.0169$). The difference between the 9.70-GHz CW group ($M = 186.7062$, $SD = 84.6619$) and the sham-exposed group is 7.1%, while the decrease in the 970-MHz CW group ($M = 186.3898$, $SD = 99.6233$) is about 6.9%.

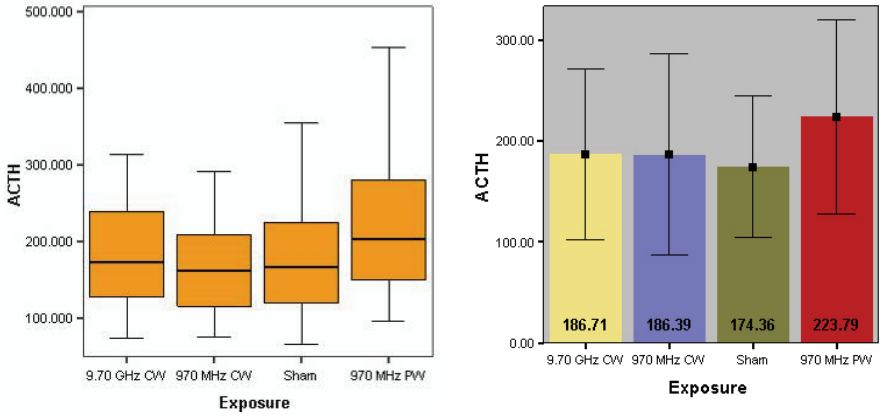

Figure B.9: Descriptive analysis of ACTH (pg/ml) in the four groups after 11 month of exposure.

It is worthwhile to investigate these findings on their statistical significance, using a Dunnett test.

In all cases, the difference between the exposed groups and the sham-exposed group is not statistically significant ($p = 0.091$, 0.909 and 0.915 for 970-MHz PW, 9.70-GHz CW and 970-MHz CW, respectively).

B.10. Corticosterone P3

After 11 month of continuous exposure (2 hours/day) the concentration of the stress hormone corticosterone (ng/ml) in the peripheral blood shows a relatively high increase (20.6%) in the 970-MHz PW group compared to the sham-exposed group (Figure B.10).

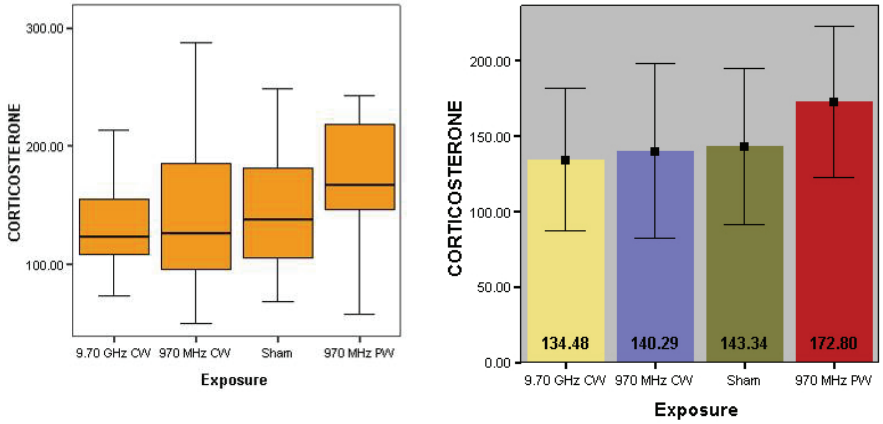

Figure B.10: Corticosterone concentrations in peripheral blood in the 4 groups of rats after 11 months of exposure.

The 970-MHz CW group and the 9.70-GHz CW group present corticosterone concentrations which are beneath the level of the sham-exposed group.

The mean value of the corticosterone concentration in the 970-MHz PW group (M = 172.80, SD = 50.32) is 20.6% higher than in the sham-exposed group (M = 143.34, SD = 53.78).

The mean value of the corticosterone level in the 970-MHz CW group (M = 140.29, SD = 57.77), represents a 2.1% decrease compared to the mean value in sham-exposed group; the corticosterone level in the 9.70-GHz CW group (M = 134.48, SD = 47.27), is 6.2% decreased related to the sham-exposed group.

A Dunnett test is performed to evaluate these changes in terms of statistical significance. The equality of the variances in all populations is verified and confirmed by the Levene test ($p > 0.05$).

The Dunnett test reveals no statistically significant differences between exposed groups and the sham-exposed group ($p = 0.082$, 0.847, 0.992 for 970 MHz PW, 9.70 GHz CW and 970 MHz CW, respectively).

B.11. Haematocrit P3

The results of the haematocrit value (%) after a period of eleven months of continuous exposure during two hours a day are graphically represented in Figure B.11.

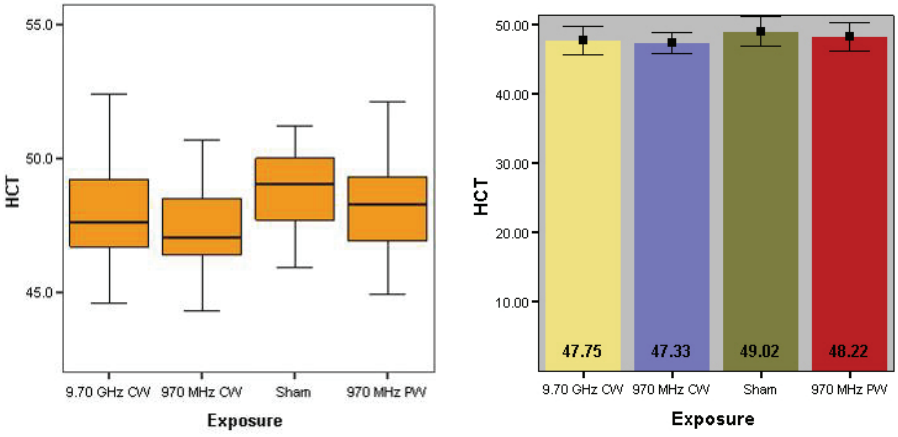

Figure B.11: Haematocrit value after 11 months of continuous exposure in the 4 groups of rats.

All the exposed groups have a decreased haematocrit value comparing to the sham-exposed group. The 970-MHz CW group (M = 47.33, SD = 1.59) differs to a larger extent (3.4%) in relation to the sham-exposed group (M = 49.02, SD = 2.10), followed by the 9.70-GHz CW group (M = 47.75, SD = 2.07) whose mean value is 2.6% lower.

The haematocrit value of the 970-MHz PW (M = 48.22, SD = 1.99) is 1.6% lower than the value of the sham-exposed group. This result is not statistically significant (p = 0.277). The haematocrit values of the 970-MHz CW group and the 9.70-GHz CW group differ in a statistically significant way from that of the sham-exposed group, with p-values of 0.003 and 0.037 respectively.

B.12. Synopsis

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 9.9%	- 0.9%	+ 3.6%
Monocytes	+ 13.0%	+ 8.8%	+ 9.6%
Erythrocytes (RBC)	- 1.3%	- 0.1%	- 0.6%
Haemoglobin	- 1.3%	0.0%	- 0.6%
Haematocrit	- 3.4%*	- 1.6%	- 2.6%*
MCV	- 1.8%*	- 1.1%	- 1.4%
MCHC	+ 2.7%*	+ 2.0%*	+ 2.3%*
Retic#	- 2.5%	- 0.8%	- 12.9%*
ACTH	+ 6.9%	+ 28.4%**	+ 7.1%
Cortico	- 2.1%	+ 20.6 %**	- 6.2%
Eosinophils	- 11.9%	- 11.5%	- 8.0%

Table B.1: Parameters presenting a statistically significant difference according to Dunnett (*) or only Student (**), compared to the sham-exposed group, after 11 months of exposure.

Appendix C: Statistical Analysis of Period 4 (P4)

In this chapter we analyze the blood parameters of 14-months-exposed rats on their statistical significance in relation to the sham-exposed rats.

The ANOVA shows significant differences ($p < 0.05$) in the means of the following parameters: leucocytes, haematocrit, reticulocytes and nearly the erythrocytes ($p = 0.052$).

Though, a result which only slightly gets over the artificially chosen significance level of 0.05 can not be neglected just like that. For example, a p-value equal to 0.052 means that the result could be only due to chance in 5.2% of the cases. This still implies that the result is not attributable to chance in 94.8% of the cases. Absence of a statistically significant result does not mean that there is no effect at all due to the independent variable.

C.1. Monocytes P4

After 14 months of exposure, we still observe an increase in monocytes in all the exposed groups compared to the sham-exposed group (Figure C.1). The rise in the 9.70-GHz CW group is the most obvious (16.6%), followed by the 970-MHz PW group (14.1%). The relative increase in the 970-MHz CW group in relation to the sham-exposed group is 9.0%.

With $p = 0.024$, the Levene test indicates that the sample variances are not equal and therefore, a Dunnett T3 test adapted to non-equal variances is performed. The normality of the distribution has been verified and confirmed.

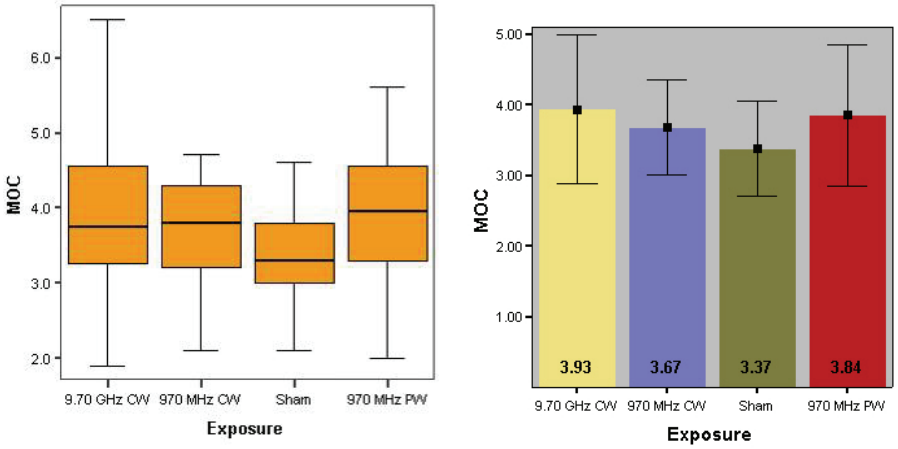

Figure C.1: Descriptive analysis of monocyte count for different exposure types after a 14-month exposure period.

The mean difference of 0.560 between the sham-exposed group (M = 3.369, SD = 0.673) and the 9.70-GHz CW group (M = 3.9290, SD = 1.0600) is found to be not statistically significant (p = 0.123).

The comparison of the mean value for the monocyte count of the 970-MHz PW exposed rats (M = 3.843, SD = 1.0046) with this of the sham-exposed rats was also found to be statistically insignificant (p = 0.224), just like the mean difference of 0.3030 between the sham-exposed group and the 970-MHz CW exposed group (M = 3.6720, SD = 0.6681) (p = 0.424).

C.2. Haemoglobin P4

The box plot representation related to a 14-month exposure period shows the descriptive statistics related to the haemoglobin concentration. This is depicted in Figure C.2. Like in the former exposure period of 11 months, only a marginally increase (nearly 2%) is found between the exposed groups and the sham-exposed group. The mean haemoglobin concentration in the 9.70-GHz CW group (M = 15.761, SD = 0.7020), the 970-MHz CW exposed group (M = 15.6860, SD = 0.6550) and the 970-MHz PW group (M = 15.6790, SD = 0.5500) do not differ significantly from the sham-exposed group (M = 15.390, SD = 0.5627) with p = 0.068, p = 0.174, and p = 0.197, respectively.

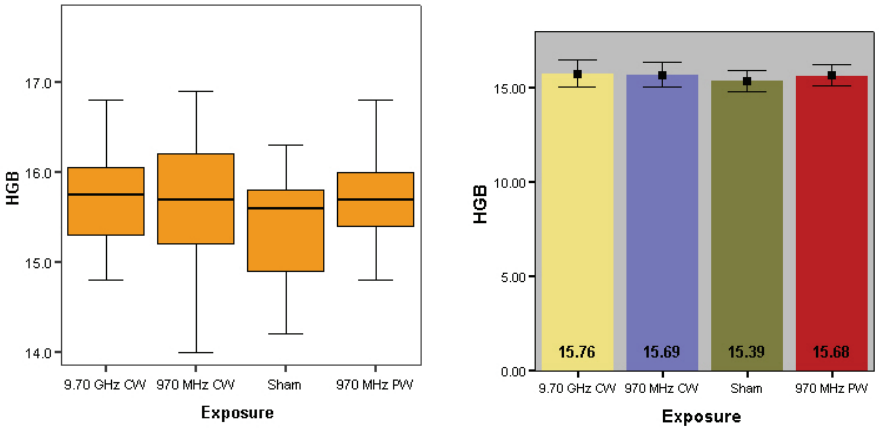

Figure C.2: Descriptive analysis of haemoglobin concentration in the four groups after 14-month exposure.

C.3. MCHC P4

Figure C.3 shows the MCMC parameter, expressed in g/dl, in the four groups of rats after a period of fourteen months of continuously exposure. One can observe a nearly equal value in all the groups. The 970-MHz PW group (M = 33.507, SD = 0.6012), the 9.70-GHz CW group (M = 33.461, SD = 0.5946) and the 970-MHz CW group (M = 33.080, SD = 0.4958) do not differ significantly from the sham-exposed group (M = 33.352, SD = 0.7936): no statistically significant difference could be detected at the 0.05 level.

With p = 0.020, the Levene test indicates that the sample variances are not equal and therefore, a Dunnett T3 test adapted to non-equal variances is performed. The normality of the distribution has also been tested before.

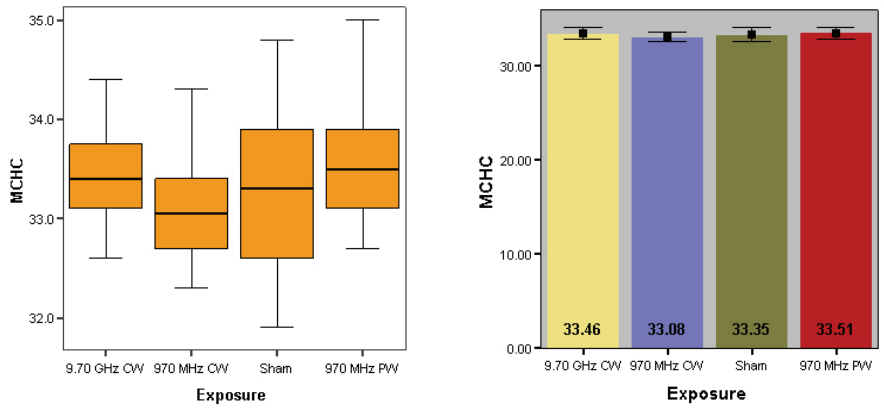

Figure C.3: Descriptive analysis of mean corpuscular haemoglobin concentration in the four groups of rats after 14-month exposure.

C.4. MCV P4

After fourteen month of continuous exposure, 2 hours a day, one can nearly observe any alterations in the four exposed groups (Figure C.4). The 970-MHz PW group (M = 54.225, SD = 1.4549), the 970-MHz CW group (M = 53.670, SD = 1.4010) and the 9.70-GHz CW group (M = 53.400, SD = 2.0072) practically do not differ from the sham-exposed group (M = 53.679, SD = 1.7060). No statistically significant difference was found for $p < 0.05$. The homogeneity of variances and the normality of the distribution have been tested before performing the Dunnett test.

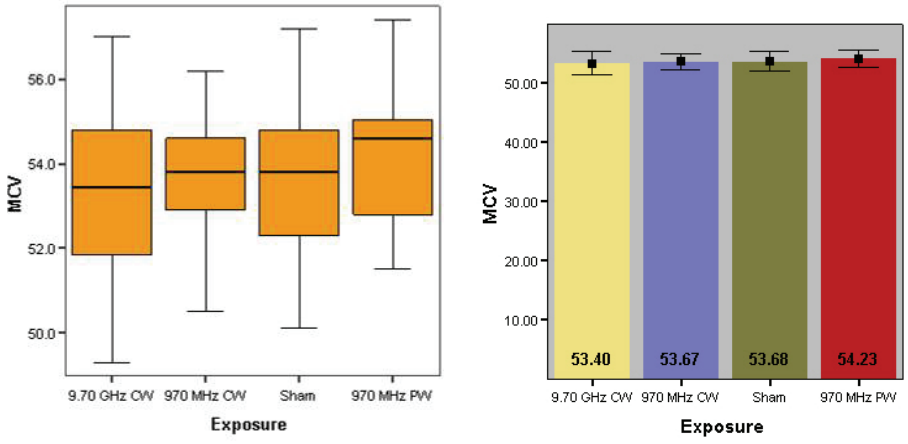

Figure C.4: Mean corpuscular volume (fl) after 14 months of exposure in the 4 groups of rats.

C.5. Reticulocytes P4

After fourteen months of exposure, all the exposed groups present higher values than the sham-exposed group (Figure C.5).

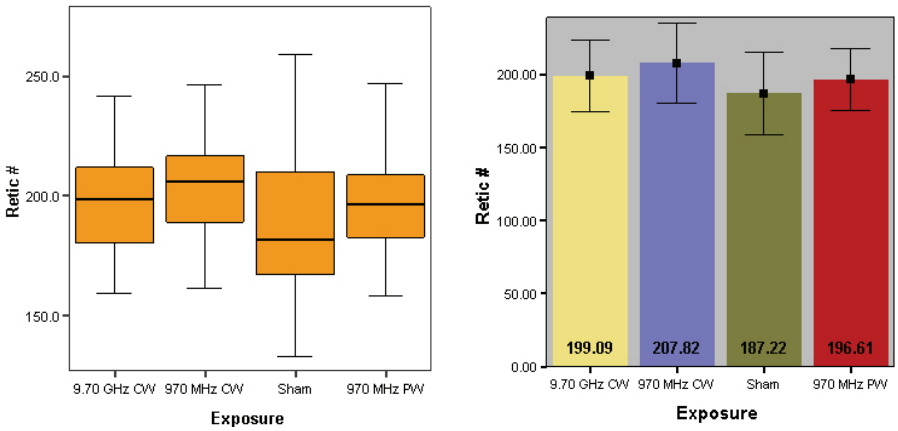

Figure C.5: Reticulocyte count in the 4 groups of rats after 14 months of exposure.

The homogeneity of variances and the normality of the distribution have been tested before performing a Dunnett test.

The reticulocyte count in the 970-MHz CW group (M = 207.817, SD = 27.5306) is 11.0% higher than in the sham-exposed group (M = 187.224, SD = 28.244). This is a statistically significant difference (p = 0.008).

The reticulocyte count in the 9.70-GHz CW group (M = 199.086, SD = 24.7481) is 6.3% higher than in the sham-exposed group. The mean difference between those two groups is not statistically significant (p = 0.202).

The 970-MHz PW group (M = 196.607, SD = 21.2956) presents a mean reticulocyte count which is 5.0% higher than the mean value in the sham-exposed group. This difference is not marked enough to be statistically different (p = 0.386).

C.6. Erythrocytes (RBC) P4

The results of the red blood cell count after a period of fourteen months continuous exposure during two hours a day are graphically represented in Figure C.6. The figures have to be multiplied by 10^6 and stand for the number of erythrocytes per µl blood. As shows Figure C.6, the situation is totally different compared to the former exposure periods. Indeed, after fourteen months of exposure, all exposed groups present a higher RBC count than the sham-exposed group.

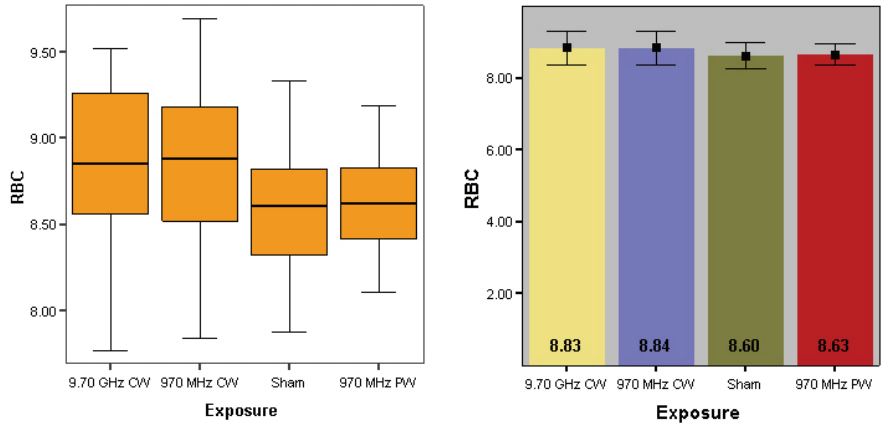

Figure C.6: Red blood cell count after 14 months of continuous exposure in the 4 groups of rats.

The mean difference between the 970-MHz CW exposed group (M = 8.8362, SD = 0.46912) and the sham-exposed group (M = 8.6038, SD = 0.37024) is statistically insignificant (p = 0.083). The RBC count in the 970-MHz CW group is 2.7% higher than in the sham-exposed group.

The mean difference between the 9.70-GHz CW exposed group (M = 8.8286, SD = 0.46883) and the sham-exposed group is also statistically insignificant (p = 0.101) and represents a 2.6% increase compared to the RBC count in the sham-exposed group.

The RBC count in the 970-MHz PW (M = 8.6329, SD = 0.29502) group does not differ significantly (+ 0.3%) from the RBC count in the sham-exposed group (p = 0.987).

C.7. Leucocytes (WBC) P4

The leucocyte count is expressed in 10^3 cells per µl. Figure C.7 shows the WBC count after the first period of a 14-month continuous exposure at a rate of two hours per day.

Unlike the former period of eleven months of exposure, statistically significant differences between exposed groups and sham-exposed group were found on the basis of a Dunnett test.

The homogeneity of variances (Levene's test with p = 0.711) and the normality of the distribution have been tested before performing the Dunnett test.

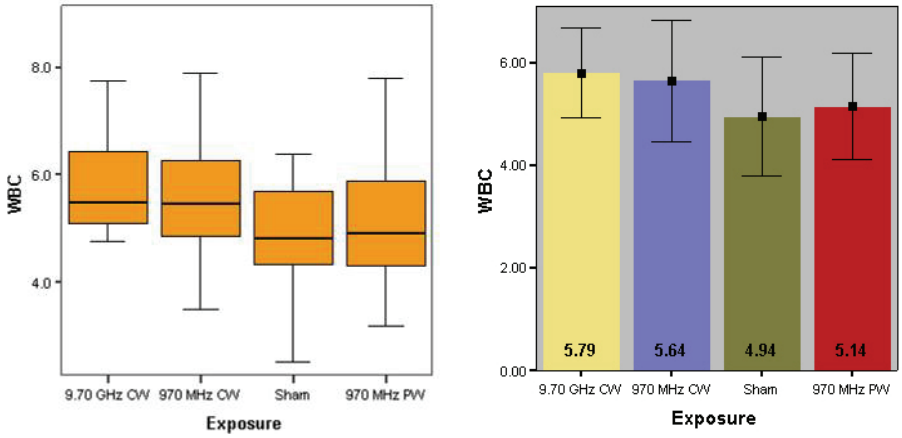

Figure C.7: White blood cell count related to the four groups of rats, after 14 months of exposure.

The white blood cell count in the 9.70-GHz CW group (M = 5.788, SD = 0.8724) is 17.2% higher (p = 0.012) than in the sham-exposed group (M = 4.940, SD = 1.1591), whereas it is 14.1 % higher in the 970-MHz CW group (M = 5.637, SD = 1.1780), (p = 0.039). The mean difference (0.697) between the 970-MHz PW (M = 5.138, SD = 1.0437) group and sham-exposed group is not statistically significant (p = 0.830). The WBC count in the 970-MHz PW group is 4.0% higher than in the sham-exposed group.

C.8. Eosinophils P4

There is a main difference between the sham-exposed group and the real exposed groups in a sense that the eosinophils value is the most elevated for the non-exposed rats (Figure 3.8). This is a similar finding as in the former periods. The homogeneity of variances and the normality of the distribution have been tested before performing a Dunnett test.

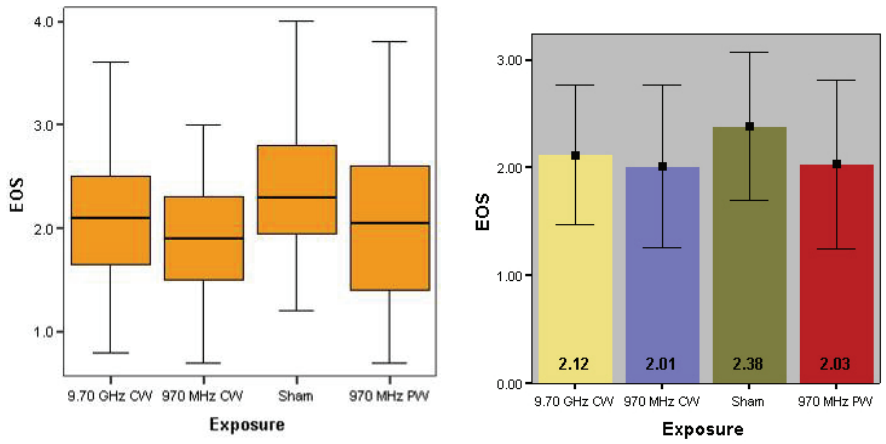

Figure C.8: Eosinophils for different exposure types after a 14-month exposure period.

The eosinophil count in the peripheral blood of the 970-MHz CW exposed group ($M = 2.0100$, $SD = 0.7558$) is 15.6% lower ($p = 0.129$) than in the sham-exposed group ($M = 2.3820$, $SD = 0.6864$) after fourteen months of exposure; the 970-MHz PW exposed group ($M = 2.0290$, $SD = 0.7831$) shows a decrease of 14.8% ($p = 0.168$) compared to the sham-exposed rats, and the 9.70-GHz CW exposed group ($M = 2.1180$, $SD = 0.6464$) a decrease of 11.1% ($p = 0.380$). Similar to the former considered period of 11 months of exposure, the results of the statistical analyses indicate that the three types of exposure are unable to induce a significant variation ($p < 0.05$).

C.9. ACTH P4

The descriptive statistical analysis graphically depicted in Figure C.9 displays the ACTH (pg/ml) concentration in the peripheral blood of the four groups of rats after fourteen months of exposure. Two out of the four groups show an increased ACTH concentration compared to the sham-exposed group: one can observe a 19.2% higher ACTH concentration in the

970-MHz PW group and a 1.1% higher concentration in the 970-MHz CW group. The 9.70-GHz CW group shows a 3.8% higher ACTH concentration than the sham-exposed group.

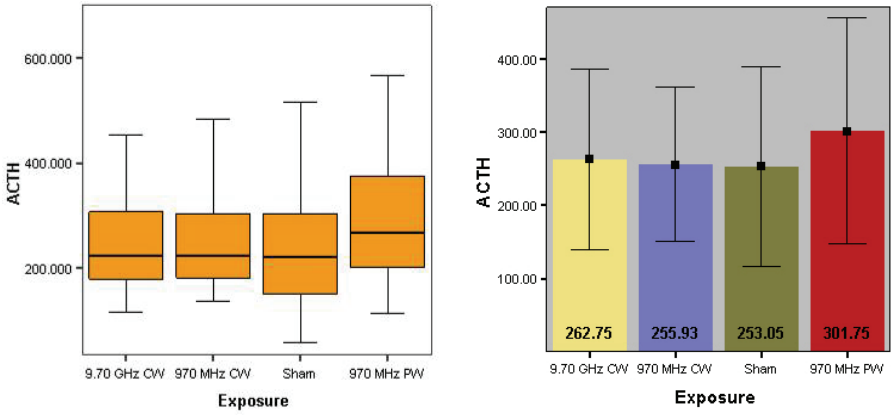

Figure C.9: Descriptive analysis of ACTH (pg/ml) in the four groups after 14 month of exposure.

The mean difference (48.6948) between the 970-MHz PW group (M = 301.7450, SD = 154.7540) and the sham-exposed group (M = 253.05020, SD = 136.0161) is not statistically significant (p = 0.361) according to the Dunnett test. The mean difference between the 970-MHz CW group (M = 255.3255, SD = 105.4746) and the sham-exposed group is also not statistically significant (p = 1.000), nor is the mean difference between 9.70-GHz CW (M = 262.7510, SD = 122.9452) and the sham-exposed group (p = 0.985).

C.10. Corticosterone P4

The 970-MHz CW group and the 9.70-GHz CW group present corticosterone concentrations (ng/ml) which are beneath the level of the sham-exposed group (Figure C.10). A Dunnett T3 test (Levene's test with

p = 0.001) is performed to evaluate these changes in terms of statistical significance (p < 0.05).

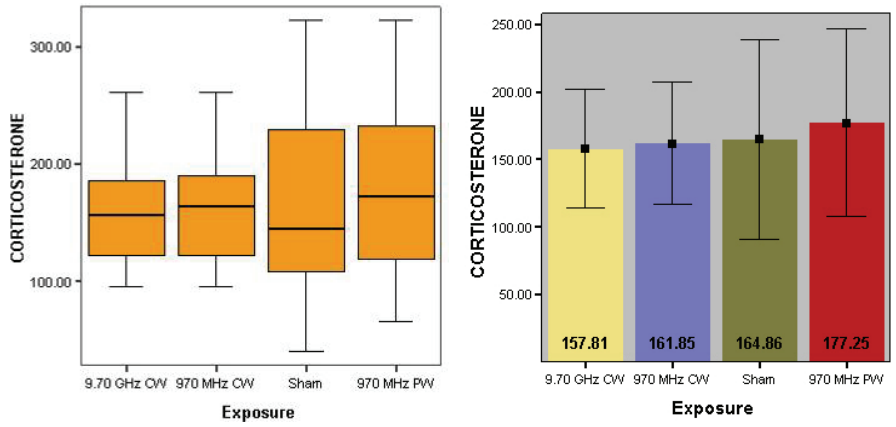

Figure C.10: Corticosterone concentrations in peripheral blood in the 4 groups of rats after 14 months of exposure.

The mean value of the corticosterone concentration in the 970-MHz PW group (M = 177.25, SD = 69.38) is 7.5% higher than in the sham-exposed group (M = 164.86, SD = 74.28). The Dunnett test reveals no statistically significant difference (p = 0.985) between the corticosterone values in the 970-MHz PW group compared to the sham-exposed group.

The mean value of corticosterone in the 970-MHz CW group (M = 161.85, SD = 45.49) shows a 1.8% decrease compared to the sham-exposed group and has to be considered as statistically not significant (p = 1.000).

The mean value of corticosterone in the 9.70-GHz CW group (M = 157.81, SD = 43.96) shows a 4.3% decrease in comparison to the sham-exposed group; this difference is not statistically significant (p = 0.998).

C.11. Haematocrit P4

The results of the haematocrit value (%) after a period of fourteen months of continuous exposure during two hours a day are graphically represented in Figure C.11. All exposed groups have an increased haematocrit value comparing to the sham-exposed group.

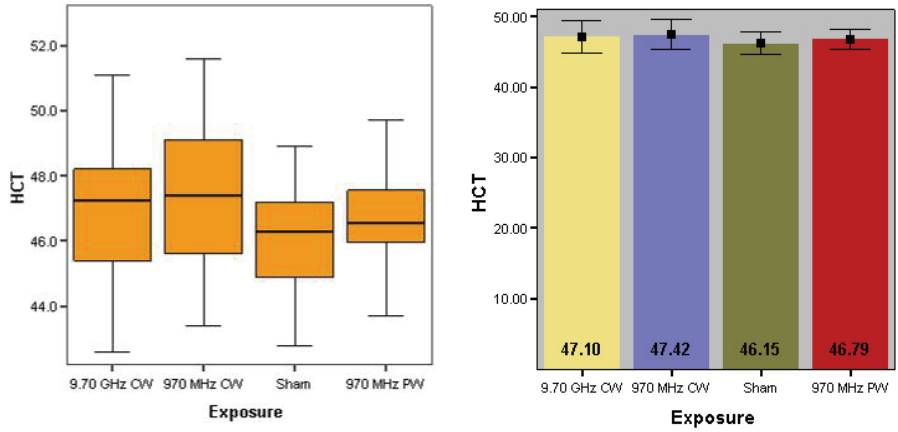

Figure C.11: Haematocrit value after 14 months of continuous exposure in the 4 groups of rats.

The 970-MHz CW group (M = 47.42, SD = 2.12) differs to a larger extent (+2.7%) in relation to the sham-exposed group (M = 46.15, SD = 1.59), followed by the 9.70-GHz CW group (M = 47.10, SD = 2.28), which haematocrit value is 2.1 % increased compared to the sham-exposed group. The haematocrit value of the 970-MHz PW (M = 46.79, SD = 1.40) is 1.4 % increased compared to the value of the sham-exposed group.

The Dunnett test reveals that compared to the sham-exposed group, the haematocrit value in the 970-MHz CW group differs significantly (p = 0.032). No significant difference is detected between the 9.70-GHz CW group and the sham-exposed group (p = 0.145). Neither a significant

difference between the 970-MHz PW group and the sham-exposed group is observed (p = 0.434).

C.12. Synopsis

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 14.1%*	+ 4.0%	+ 17.2%*
Monocytes	+ 9.0%	+ 14.1%**	+ 16.6%**
Erythrocytes (RBC)	+ 2.7%**	+ 0.3%	+ 2.6%**
Haemoglobin	+ 1.9%	+ 1.9%	+ 2.4%**
Haematocrit	+ 2.7%*	+ 1.4%	+ 2.1%
MCV	- 0.02%	+ 1.0%	- 0.5%
MCHC	- 0.8%	+ 0.5%	+ 0.3%
Retic#	+ 11.0%*	+ 5.0%	+ 6.3%
ACTH	+ 1.1%	+ 19.2%	+ 3.8%
Cortico	- 1.8%	+ 7.5%	- 4.3%
Eosinophils	- 15.6%	- 14.8%	- 11.1%

Table 5.1: Parameters presenting a statistically significant difference according to Dunnett (*) or only Student (**), compared to the sham-exposed group, after 14 months of exposure.

Appendix D: Statistical Analysis of Period 5 (P5)

In this chapter we analyze the blood parameters of 18-months-exposed rats on their statistical significance in relation to the sham-exposed rats.

The ANOVA shows significant differences in the means of the following parameters: leucocytes, mean corpuscular haemoglobin concentration, neutrophils and lymphocytes, and reticulocytes.

D.1. Monocytes P5

After 18 months of exposure, we still observe an increase in monocytes in all the exposed groups compared to the sham-exposed group (Figure D.1). This is a similar tendency as in the former exposure periods.

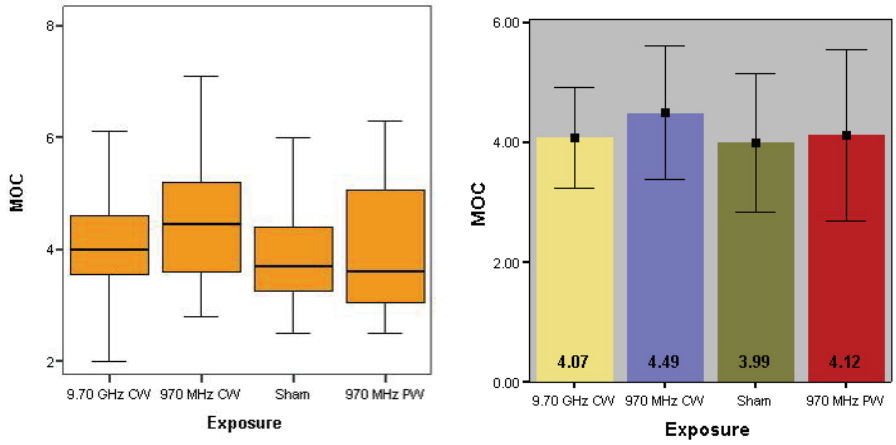

Figure D.1: Descriptive analysis of monocyte count for different exposure types after an 18-month exposure period.

The rise in the 970-MHz CW group is the most obvious (12.5%), followed by the 970-MHz PW group (3.3%). The relative increase of the 9.70-GHz CW group in relation to the sham-exposed group is 2.0%.

The Levene test indicates that the sample variances between all groups are equal. The normality of the distributions has been verified and confirmed.

The mean difference of 0.500 between the sham-exposed group (M = 3.99, SD = 1.154) and the 970-MHz CW group (M = 4.49, SD = 1.112) is found to be statistically not significant (p = 0.357).

The mean value for the monocyte count of the 970-MHz PW exposed rats (M = 4.12, SD = 1.435) compared with those of the sham-exposed rats, do not elucidate a statistically significant difference (p = 0.959).

The mean difference of 0.303 between the sham-exposed group and the 9.70-GHz CW group (M = 4.07, SD = 0.848), is not statistically significant (p = 0.987).

D.2. Haemoglobin P5

The box plot representation related to 18 months of exposure shows the descriptive statistics related to the haemoglobin concentration. This is depicted in Figure D.2.

With p = 0.603, the Levene test indicates that the sample variances between the groups are equal. The normality of the distribution has been verified and confirmed.

Applying the Dunnett test learns that the mean difference of 0.505 between the sham-exposed group (M = 16.126, SD = 1.6426) and the 9.70-GHz CW

group (M = 16.631, SD = 1.4327), equivalent with a 3.1% increase, is not statistically significant (p = 0.550).

The comparison of the mean value for the haemoglobin concentration of the 970-MHz PW exposed rats (M = 15.871, SD = 1.4994) with those of the sham-exposed rats revealing a 1.6% decrease compared to the sham-exposed group, is found to be statistically insignificant (p = 0.905).

The mean difference of 0.5760 between the sham-exposed group and the 970-MHz CW group (M = 15.550, SD = 2.0217), equivalent with a 3.6% decrease in the 970-MHz CW group, is not statistically significant (p = 0.527).

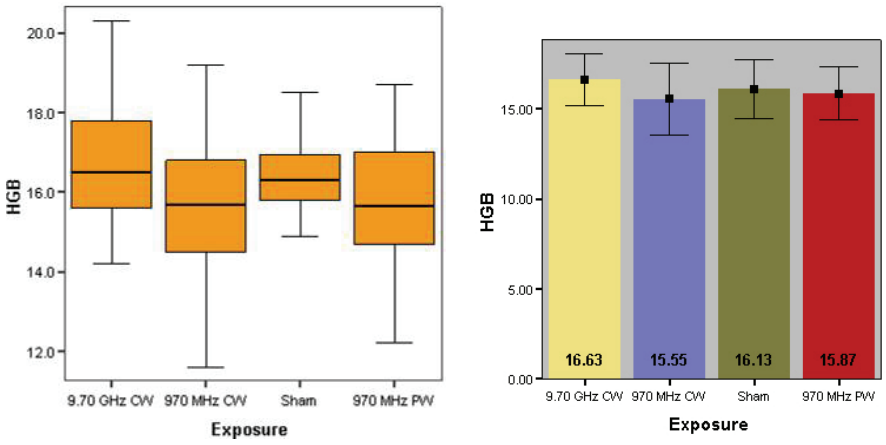

Figure D.2: Descriptive analysis of haemoglobin concentration in the four groups after 18-month exposure.

D.3. MCHC P5

Figure D.3 shows the MCMC parameter, expressed in g/dl, in the four groups of rats after a period of eighteen months of continuous exposure, two hours a day.

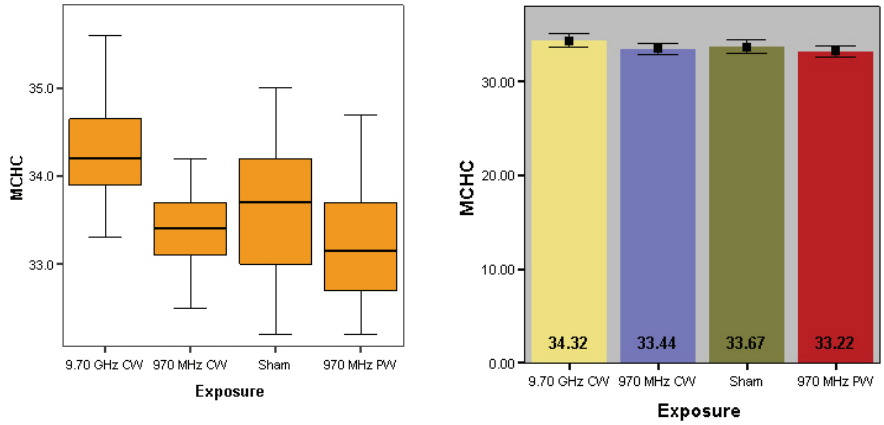

Figure D.3: Descriptive analysis of mean corpuscular haemoglobin concentration in the four groups of rats after 18-month exposure.

With p = 0.444, the Levene test indicates that the sample variances between the groups are equal. The normality of the distributions has been verified and confirmed.

The mean difference of 0.649 between the 9.70-GHz CW group (M = 34.322, SD = 0.7062) and the sham-exposed group (M = 33.673, SD = 0.7454) is statistically significant at a 0.05 level (p = 0.002). This represents an increase of 1.9%.

The mean difference of 0.5760 between the sham-exposed group and the 970-MHz PW group (M = 33.221, SD = 0.6014), equivalent with a 1.3% decrease in the 970-MHz PW group, is statistically significant (p = 0.049).

The Dunnett test compared the mean value for the MCHC parameter of the 970-MHz CW exposed rats (M = 33.444, SD = 0.5469) with those of the sham-exposed rats. This comparison, revealing a 0.7% decrease compared to the sham-exposed group, is found to be statistically insignificant (p = 0.550).

D.4. MCV P5

After eighteen months of continuous exposure, 2 hours a day, one can nearly observe any alterations in the four exposed groups (Figure D.4). The differences in comparison with the sham-exposed group are of the order of 1%. No statistical differences have been found.

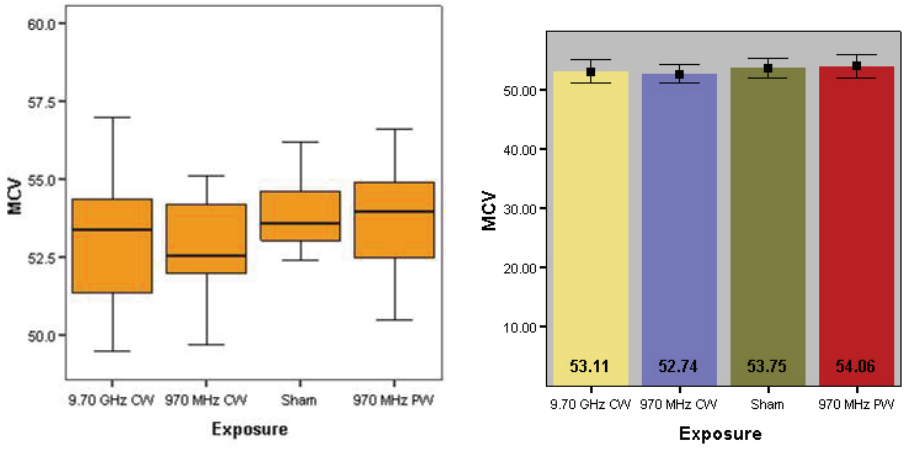

Figure D.4: Mean corpuscular volume (fl) after 18 months of exposure in the 4 groups of rats.

With p = 0.451, the Levene test indicates that the sample variances between groups are equal. The normality of the distributions has been verified and confirmed.

The mean difference of 1.004 between the sham-exposed group (M = 53.748, SD = 1.6951) and the 970-MHz CW group (M = 52.744, SD = 0.1.5689) is not statistically significant at a 0.05 level (p = 0.189). This represents a decrease of 1.9% in the 970-MHz CW group.

Dunnett's test compared also the mean value for the MCV parameter of the 9.70-GHz CW exposed rats (M = 53.115, SD = 1.9868) with those of the sham-exposed rats. This comparison, revealing a 1.2% decrease compared to the sham-exposed group, is found to be statistically insignificant (p = 0.457).

The mean difference of 0.315 (54.063 – 53.748) between the sham-exposed group and the 970-MHz PW group (M = 54.063, SD = 2.0056), equivalent with a 0.6% increase in the 970-MHz PW group, is statistically not significant (p = 0.881).

D.5. Reticulocytes P5

All the exposed groups present higher values than the sham-exposed group (Figure D.5).

The normality of the distributions has been evaluated and confirmed. The variances however are not equal conform the Levene test (p = 0.027). Therefore, a Dunnett T3 test has been performed.

The reticulocyte count in the 970-MHz PW group (M = 238.629, SD = 58.4486) is 15.5% higher than in the sham-exposed group (M = 206.5965, SD = 39.5706). This is not a statistically significant difference at a 0.05 significance level (p = 0.162).

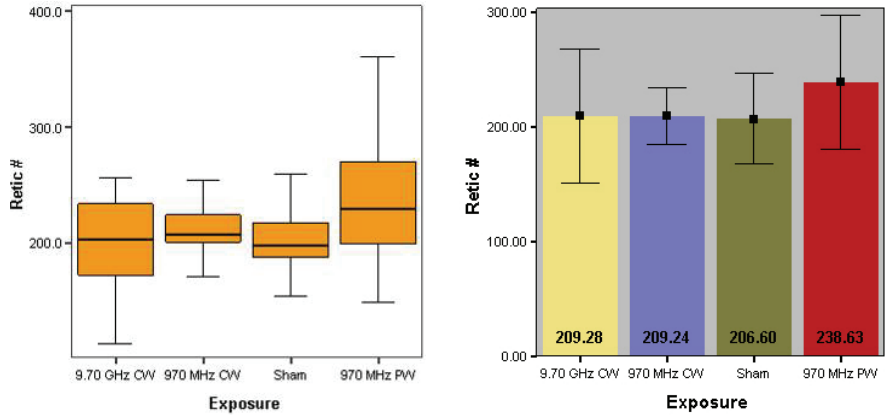

Figure D.5: Reticulocyte count in the 4 groups of rats after 18 months of exposure.

The reticulocyte count in the 9.70-GHz CW group (M = 209.282, SD = 58.1685) is 1.3% higher than in the sham-exposed group. The mean difference between those two groups is not statistically significant (p = 1.000).

The 970-MHz CW group (M = 209.232, SD = 25.0179) has an almost identical reticulocyte count than the 9.70-GHz CW group and is not statistically different either (p = 1.000).

D.6. Erythrocytes (RBC) P5

The results of the red blood cell count after a period of eighteen months continuous exposure during two hours a day are graphically represented in Figure D.6. The figures have to be multiplied by 10^6 and stand for the number of erythrocytes per µl blood.

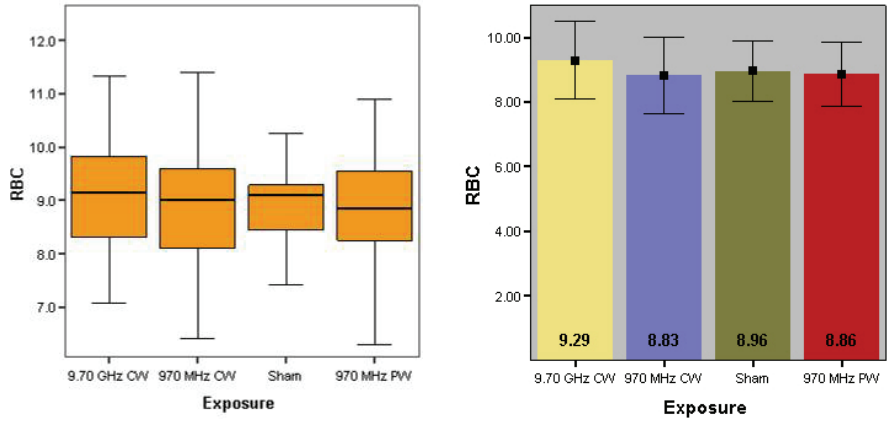

Figure D.6: Red blood cell count after 18 months of continuous exposure in the 4 groups of rats.

The normality of the distributions and the equality of variances between compared groups have been evaluated and confirmed before applying a Dunnett test.

The mean difference between the 9.70-GHz CW exposed group (M = 9.287, SD = 1.2074) and the sham-exposed group (M = 8.960, SD = 0.9365) is not statistically significant (p = 0.557). The RBC count in the 9.70-GHz CW group is 3.6% higher than in the sham-exposed group.

The mean RBC count of the 970-MHz PW and the 970-MHz CW group is beneath the mean level of the sham-exposed group. The mean difference between the 970-MHz PW exposed group (M = 8.858, SD = 0.9824) is 1.1% lower than in the sham-exposed group. This difference is not statistically significant (p = 0.976).

The RBC count in the 970-MHz CW (M = 8.833, SD = 1.1827) group is 1.4% decreased in comparison with the sham-exposed group.

In these, the 970-MHz CW group does not differ significantly from the sham-exposed group (p = 0.964).

D.7. Leucocytes (WBC) P5

The leucocyte count is expressed in 10^3 cells per µl. Figure D.7 shows the WBC after the first period of an 18 month continuous exposure at a rate of two hours per day.

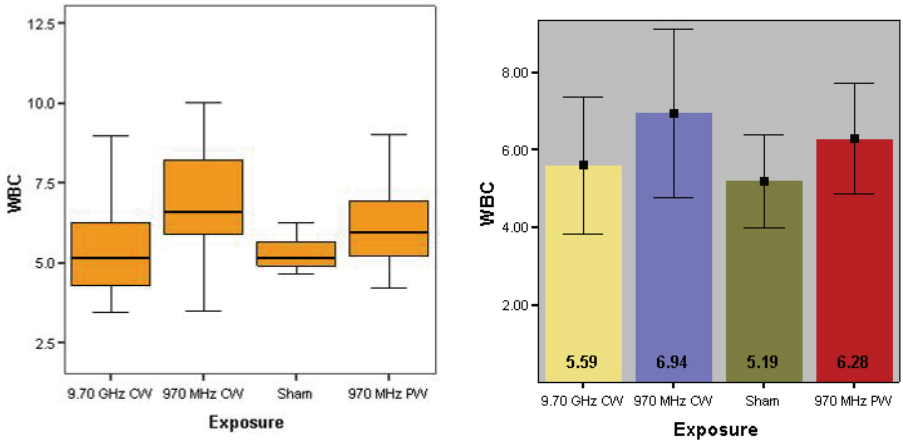

Figure D.7: White blood cell count related to the four groups of rats, after 18 months of exposure.

Similarly to the former period of fourteen months of exposure, the mean values of white blood cell count are higher in the exposed groups compared to the sham-exposed group after eighteen months of exposure. Statistically significant differences between exposed groups and the sham-exposed group are found on the basis of a Dunnett test.

The normality of the distributions and the equality of variances between compared groups have been evaluated and confirmed.

We find a statistically significant difference ($p = 0.002$) between the WBC count in the 970-MHz CW group ($M = 6.939$, $SD = 2.1715$) compared to the sham-exposed group ($M = 5.192$, $SD = 1.1952$). The increase in the 970-MHz CW group is 33.6%.

The WBC count is 21.0% higher in the 970-MHz PW group ($M = 6.283$, $SD = 1.4248$), than in the sham-exposed group. This is found to be nearly statistically different ($p = 0.055$).

The mean difference of 0.399 between the 9.70-GHz CW ($M = 5.591$, $SD = 1.7696$) group and sham-exposed group is not statistically significant ($p = 0.712$). The WBC count in the 9.70-GHz CW group is 7.7% higher than in the sham-exposed group.

D.8. Eosinophils P5

Compared to the former exposure periods, there is a main difference between the sham-exposed group and the real exposed groups in a sense that the eosinophils value doesn't show the highest level in the sham-exposed group (Figure D.8).

After eighteen months of exposure, the eosinophil count in the peripheral blood of the 970-MHz CW ($M = 2.294$, $SD = 0.8461$) is 19.4 % higher ($p = 0.262$) than in the sham-exposed group ($M = 1.922$, $SD = 0.7944$). The mean value of the 970-MHz PW ($M = 2.078$, $SD = 0.6295$) group shows an increase of 8.1% compared to those in the sham-exposed rats ($p = 0.817$); the 9.70-GHz CW group displays a decrease of 2.5% ($p = 0.992$) in comparison with the sham-exposed group. Similar to the former considered period of fourteen months of exposure, the results of the statistical analyses indicate that the three types of exposure are unable to induce a significant difference at $p < 0.05$ related to the eosinophil parameter.

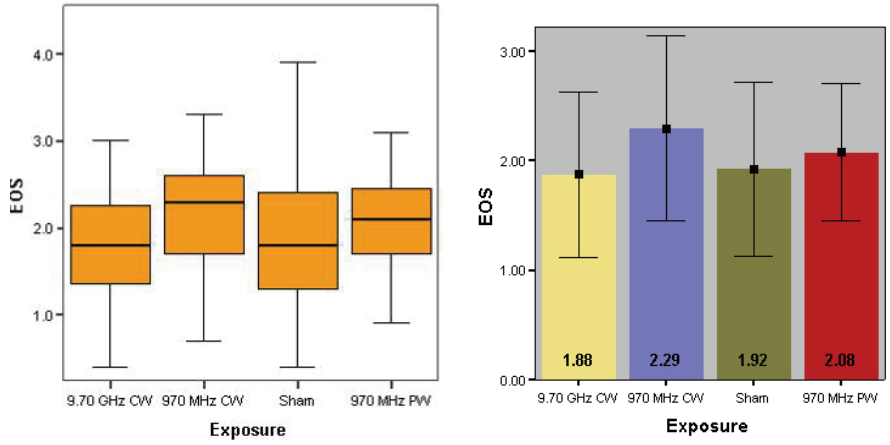

Figure D.8: Eosinophils for different exposure types after an 18-month exposure period.

D.9. ACTH P5

The descriptive statistical analysis graphically depicted in Figure D.9, displays the ACTH (pg/ml) concentration in the peripheral blood of the four considered groups of rats after fourteen months of exposure. Two out of the four groups show an increased ACTH concentration compared to the sham-exposed group, but it concerns other groups than in the former exposure period of fourteen months: one can observe a 23.2% higher ACTH concentration in the 970-MHz CW group and a 1.3% higher concentration in the 9.70-GHz CW group. The 970-MHz PW group shows a 6.9% lower ACTH concentration than the sham-exposed group.

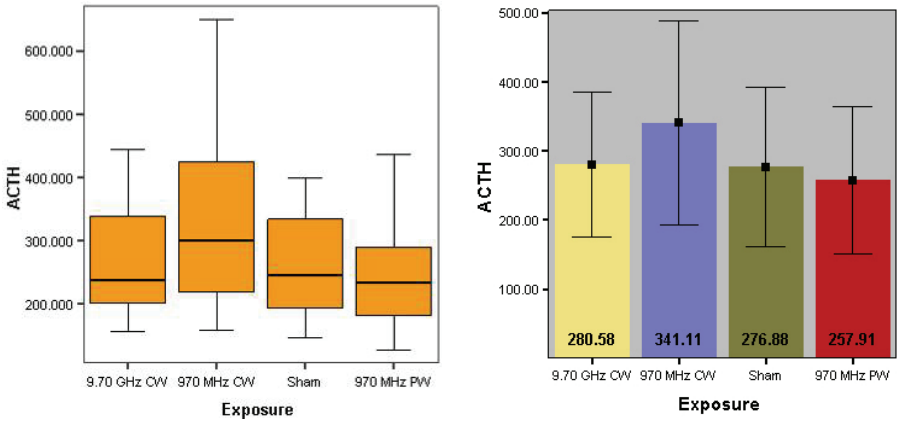

Figure D.9: Descriptive analysis of ACTH (pg/ml) in the four groups after 18 month of exposure.

The normality of the distributions and the equality of variances between compared groups have been evaluated and confirmed.

The mean difference (64.23) between the 970-MHz CW group (M = 341.108, SD = 147.742) and the sham-exposed group (M = 276.8780, SD = 115.6451) is not statistically significant (p = 0.191), when using a Dunnett test with a level of significance of $p < 0.05$. The mean difference between the 9.70-GHz CW group (M = 280.576, SD = 105.187) and the sham-exposed group is not statistically significant (p = 0.999), nor is the mean difference between the 970-MHz PW (M = 257.9123, SD = 106.2033) and the sham-exposed group (p = 0.894).

D.10. Corticosterone P5

In contrast with the former exposure period of fourteen months, all exposed groups show a lower corticosterone level than the sham-exposed group, after eighteen months of exposure (Figure D.10).

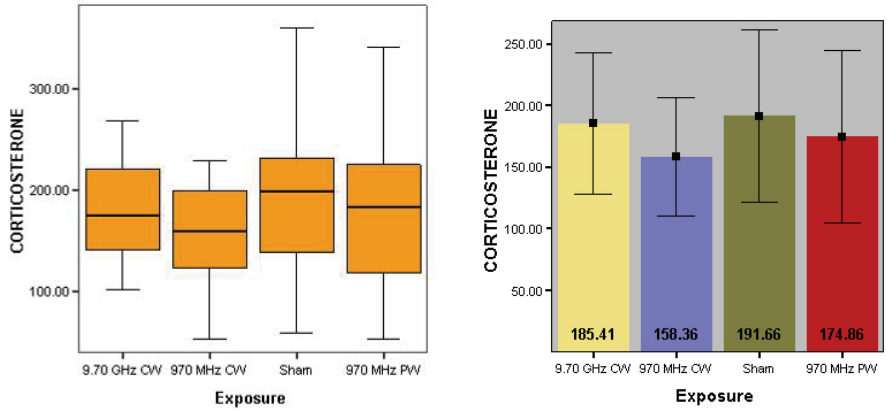

Figure D.10: Corticosterone concentrations in peripheral blood in the 4 groups of rats after 18 months of exposure.

A Dunnett test is performed to evaluate these changes in terms of statistical significance ($p < 0.05$).

The normality of the distributions and the equality of variances between compared groups have been evaluated and confirmed.

The mean value of the corticosterone concentration (ng/ml) in the 9.70-GHz CW group ($M = 185.4054$, $SD = 57.4960$) is 3.3% lower than in the sham-exposed group ($M = 191.6608$, $SD = 69.9830$). Dunnett's test reveals no statistically significant difference ($p = 0.970$) between the mean corticosterone values in the 9.70-GHz CW group compared to the sham-exposed group.

The mean value of corticosterone in the 970-MHz PW group ($M = 174.8650$, $SD = 69.9298$) is 8.8% decreased compared to the mean value in the sham-exposed group. This difference is not statistically significant ($p = 0.675$).

The mean difference between the 970-MHz CW group ($M = 158.3633$, $SD = 48.0053$) and the sham-exposed group is not statistically different ($p = 0.215$), although the ACTH value is 17.4% lower.

D.11. Neutrophils P5

For the first time, after the entire exposure period of eighteen months, statistically significant differences are observed concerning the parameter of the neutrophils. All the exposed groups have higher values than the sham-exposed group. This can be seen in Figure D.11. In general, the difference compared to the sham-exposed group is more than 25%.

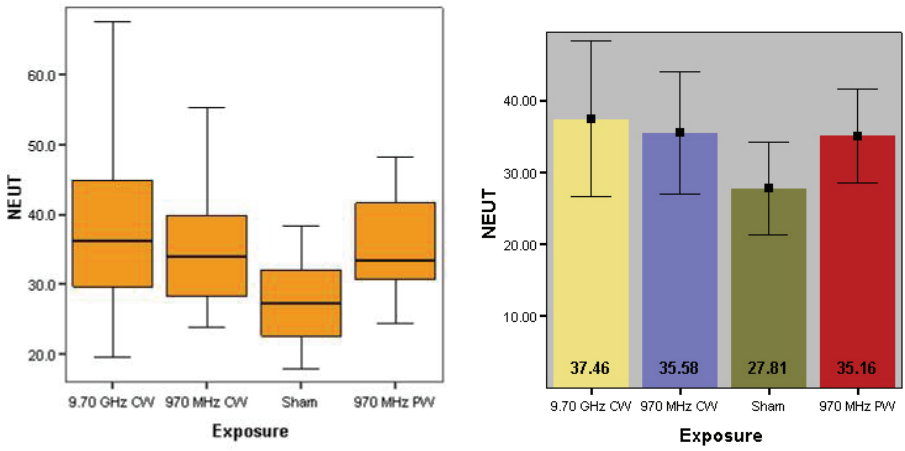

Figure D.11: Neutrophils in the 4 groups of rats after 18 months of exposure.

The Levene test for equality of variances indicates that the variances in the groups are different ($p = 0.039$). Therefore, a Dunnett T3 test is performed. The normality of the distribution has been evaluated and confirmed.

The mean value of the 9.70-GHz CW group ($M = 37.456$, $SD = 10.8583$) is 34.7% higher than the mean value of the sham-exposed group. This difference is statistically significant ($p = 0.002$).

The mean value of the 970-MHz CW group ($M = 35.583$, $SD = 8.5283$) is 27.9% higher than the mean value of the sham-exposed group. This is statistically significant ($p = 0.016$).

The mean value of the 970-MHz PW group (M = 27.812, SD = 6.5063) is 26.4% higher than the mean value of the sham-exposed group. The Dunnett T3 test reveals a difference which is statistically different too (p = 0.001).

D.12. Lymphocytes P5

For the first time, after the entire exposure period of eighteen months, statistically significant differences are observed concerning the parameter of the lymphocytes. All exposed groups have a lower mean lymphocytes count than the sham-exposed group. This can be observed in Figure D.12.

In general, the decrease amounts to 15% compared to the mean level of this parameter in the sham-exposed group.

First, the normality and the equality of the variances between the compared groups have been verified and confirmed. A Dunnett test has been applied to evaluate if the observed differences are statistically significant at a p-level of 0.05.

The mean value of the 9.70-GHz CW group (M = 56.096, SD = 11.3439) is 15.1% lower than the mean value in the sham-exposed group (M = 66.085, SD = 6.7087). This difference is statistically significant (p = 2.47 10-4).

The mean value of the 970-MHz CW group (57.500, SD = 9.1304) is 13.0% lower than the mean value of the sham-exposed group. This is a statistically significant difference (p = 0.006).

The mean value of the 970-MHz PW group (M = 58.413, SD = 7.2294) is 11.6 % lower than the mean value of the sham-exposed group and is statistically significant (p = 0.008).

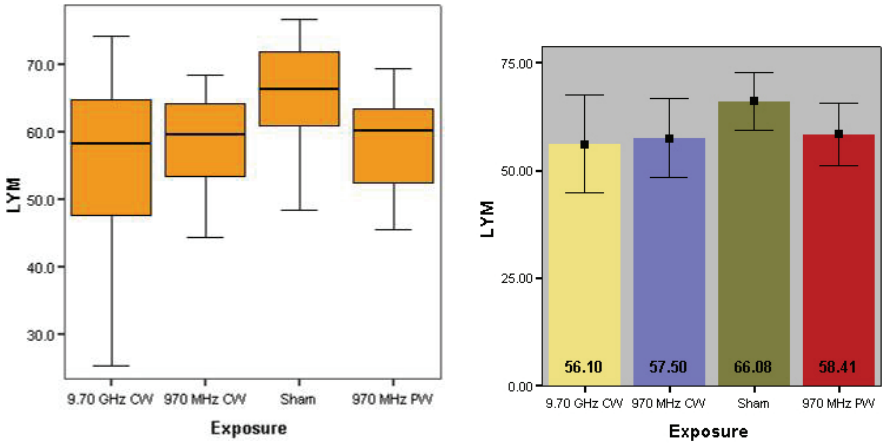

Figure D.12: Lymphocyte count in the 4 groups of rats after 18 months of exposure.

D.13. Synopsis

	970 MHz CW	970 MHz PW	9.70 GHz CW
Leucocytes (WBC)	+ 33.6%*	+ 21.0%**	+ 7.7%
Monocytes	+ 12.5%	+ 3.3%	+ 2.1%
Erythrocytes (RBC)	- 1.4%	- 1.1%	+ 3.6%
Haemoglobin	- 3.6%	- 1.6%	+ 3.1%
Haematocrit	- 3.3%	- 0.7%	+ 0.8%
MCV	- 1.9%	+ 0.6%	- 1.2%
MCHC	- 0.7%	- 1.3%*	+ 1.9%*
Retic#	+ 1.3%	+ 15.5%**	+ 1.3%
Neutrophils	+ 27.9*	+ 26.4%*	+ 34.7*
Lymphocytes	- 13.0%*	- 11.6%*	- 15.1%*
ACTH	+ 23.2%	- 6.9%	+ 1.3%
Cortico	- 17.4%	- 8.8%	- 3.3%
Eosinophils	+ 19.4%	+ 8.1%	+ 2.5%

Table 4.1: Parameters presenting a statistically significant difference according to Dunnett (*) or only Student (**), compared to the sham-exposed group, after 18 months of exposure.