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An Epidemiological Study on Low-level 21-month Microwave Exposure of Rats
(Introduction and Conclusion)

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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.

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 18th 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. Further 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.