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Chapter 3

MOBILE TELEPHONY RADIATION EFFECTS ON LIVING ORGANISMS

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Abstract

A number of serious non thermal biological effects, ranging from changes in cellular function like proliferation rate changes or gene expression changes to cell death induction, decrease in the rate of melatonin production and changes in electroencephalogram patterns in humans, population declinations of birds and insects, and small but statistically significant increases of certain types of cancer, are attributed in our days to the radiations emitted by mobile telephony antennas of both handsets and base stations. This chapter reviews briefly the most important experimental, clinical and statistical findings and presents more extensively a series of experiments, concerning cell death induction on a model biological system. Mobile telephony radiation is found to decrease significantly and non thermally insect reproduction by up to 60%, after a few minutes daily exposure for only few days. Both sexes were found to be affected. The effect is due to DNA fragmentation in the gonads caused by both types of digital mobile telephony radiation used in Europe, GSM 900MHz, (Global System for Mobile telecommunications), and DCS 1800MHz, (Digital Cellular System). GSM was found to be even more bioactive than DCS, due to its higher intensity under equal conditions. The decrease in reproductive capacity seems to be non-linearly depended on radiation intensity, exhibiting a peak for intensities higher than $200 \mu\text{W}/\text{cm}^2$ and an intensity "window" around $10 \mu\text{W}/\text{cm}^2$ where it becomes maximum. In terms of the distance from a mobile phone antenna, the intensity of this "window" corresponds under usual conditions to a distance of 20-30 cm. The importance of different parameters of the radiation like intensity, carrier frequency and pulse repetition frequency, in relation to the recorded effects are discussed. Finally, this chapter describes a plausible biophysical and biochemical mechanism which can explain the recorded effects of mobile telephony radiations on living organisms.

Keywords: mobile telephony radiation, GSM, DCS, RF, ELF, electromagnetic fields, non-ionizing electromagnetic radiation, biological effects, health effects, *Drosophila*, reproductive capacity, cell death, intensity windows.

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Introduction

As mobile telephony becomes more and more a necessary tool in our daily life enabling modern man to communicate easily with everyone at any place and any moment, serious threats arise from the exposure of all living organisms and the environment to a type of radiation unknown until now. Man made electromagnetic fields and radiations differ substantially from natural electromagnetic radiations like natural light, mainly because artificial ones are polarised, able to induce coherent forced vibrations to any electric charge in their space. All living organisms are made of cells and all cellular functions are of electrical nature, involving movements of electrical charges like clouds of free ions or charged macromolecules. Certain movements of certain type of charges within the cells induce or interrupt corresponding cellular functions. Any wrong, synchronized net movement of charge within the cell, would induce a wrong cellular function. The cell as a highly organized unit of life, has protective mechanisms against wrong cellular function, for example by activating certain genes and consequently producing certain proteins like the “heat shock” ones, made to protect the cell from excessive heat. But if the cell fails to protect itself from an external disturbance, a malfunction may start which can be transferred to a whole tissue or the whole organism. Electromagnetic fields (EMFs) are perceived by the cells as external disturbances or external stress but the cells don’t seem to have special genes to be activated for protection against electromagnetic stress. This might be the reason why in response to electromagnetic stress, cells activate heat shock genes and produce heat shock proteins very rapidly (within minutes) and at a much higher rate than for heat itself, (Weisbrot et al, 2003). It seems to be for the same reason why electromagnetic stress from mobile telephony radiation induces cell death to the reproductive cells much more than other types of external stress examined before like food deprivation or chemicals, (Panagopoulos et al 2007a). Thus it seems that cells are much more sensitive to man-made electromagnetic fields (EMFs) than to other types of stress previously known. This is probably due to the fact that man-made EMFs constitute a new and perhaps more intense type of external stress, against which, cells have not developed defensive mechanisms. If cells activate heat shock genes to protect themselves from electromagnetic stress and this happens at a much higher rate than for heat itself, this might be dangerous, since repetitive stress leading to continuous expression of heat shock genes may result to cancer induction, (French et al, 2001).

A number of biological effects induced by man-made (EMFs) and radiations of different frequencies including digital mobile telephony and microwave radiations, have already been reported and documented by many research groups. These include changes in intracellular ionic concentrations, changes in the synthesis rate of different biomolecules, changes in cell proliferation rates, changes in the reproductive capacity of animals, changes in gene expression and even DNA damage and cell death,, (Aitken et al 2005; Bawin and Adey 1976; Bawin et al. 1975; 1978; Barteri et al 2005; Belyaev et al 2005; Blackman et al 1980; 1989; Caraglia et al 2005; Diem et al 2005; Dutta et al 1984; Kwee and Raskmark 1998; Velizarov et al 1999; Magras and Xenos 2001; Xenos and Magras 2003; Panagopoulos et al 2004; 2007a; 2007b; Lai and Singh 1995; 1996; 1997; 2004; Remondini et al 2006; Nylund and Leszczynski 2006; Diem et al 2005; Salford et al 2003). At the same time, some epidemiological studies are starting more and more to indicate a connection between the use

of cellular mobile phones and certain types of cancer, (Hardell et al 2007a; Hardell et al 2006; Hardell and Hansson-Mild, 2006; Kundi 2004).

In several cases, melatonin, a hormone which controls the daily biological cycle and has an oncostatic action, produced by the epiphysis (pineal gland) in mammals, mainly during the night, is found to reduce the action of EMR exposure, but the synthesis of melatonin itself seems to be reduced by EMR, (Burch et al, 2002; Ozguner et al, 2006; Oktem et al, 2005).

Technical Characteristics of Digital Mobile Telephony Radiation

Both systems of Digital Mobile Telephony Radiation used in Europe, GSM 900 MHz and DCS 1800 MHz and also the system used in USA, GSM 1900 MHz, use different carrier frequencies, (900, 1800, and 1900 MHz respectively), but the same pulse repetition frequency of 217 Hz, (Hillebrand 2002; Clark 2001; Hyland 2000; Hamnerius and Uddmar 2000; Tisal 1998). As is obvious, the signals of Digital Mobile Telephony Radiation, combine “radio frequencies” (RF) and “extremely low frequencies” (ELF). All three systems use the “Time Division Multiple Access” (TDMA) code to increase the number of people that can simultaneously communicate with a base station. The radiation is emitted in frames of 4.615 msec duration, at a repetition rate of 217 Hz. Each frame consists of eight “time slots” and each user occupies one of them. Within each time slot the microwave radiation uses a type of phase modulation called “Gaussian Minimum Shift Keying” modulation (GMSK) to carry the information, (Tisal 1998; Hamnerius and Uddmar 2000). The transmitted frames by both handsets and base stations are grouped into multi-frames of 25 by the absence of every 26th frame. This results to an additional multi-frame repetition frequency of 8.34 Hz. Finally, handsets emit an even lower frequency at 2 Hz whenever the user is not speaking, for energy saving reasons, (“non-modulated” or “non-speaking” emission or “discontinuous transmission mode”- DTX), (Hyland 2000). Of course, when the handsets operate at DTX mode, the average emitted power is much less (about one tenth of the emitted power when they operate at “speaking” mode, (Panagopoulos et al, 2000a; 2004).

Except of the carrier frequency, another important difference between the three systems of digital mobile telephony radiation is that GSM 900MHz antennas of both mobile phones and base stations operate with double the output power than the corresponding DCS 1800MHz ones or the GSM 1900 MHz ones. GSM 900 MHz handsets operate with 2 W peak power output, while DCS 1800 MHz and GSM 1900 MHz ones operate with 1 W peak power output.

Radiation from base station antennas is almost identical to that from mobile phones of the same system (GSM or DCS), except that it is about 100 times more powerful, or to be more accurate, from several tens up to several hundred times more powerful. Thereby, effects produced by mobile phones at certain distances, can be extrapolated to represent effects from base station antennas at about 100 times longer distances. Another difference is that handset signals include one pulse per frame occupying one time slot, whereas base station signals include again one pulse per frame but this pulse may occupy 1-8 time slots depending on the number of subscribers each moment. In other words the ratio between pulse peak power and time-averaged power is usually higher for the handset signals compared to the base station signals, (Hillebrand 2002; Clark 2001; Hyland 2000; Hamnerius and Uddmar 2000; Tisal 1998).

Established Exposure Criteria for Mobile Telephony Radiations

The most stringent international exposure limits in the western world for RF radiation used by digital mobile telephony were set by the International Radiation Protection Association (IRPA) and the International Commission on Non-Ionizing Radiation Protection (ICNIRP). These criteria were established to protect biological tissue from temperature increases, (thermal effects).

The ICNIRP exposure limits are given either in terms of Radiation Intensity (Power Density) usually in mW/cm^2 , either in terms of Specific Absorption Rate (SAR) which is defined as the radiation power, absorbed by the unit mass of tissue, in W/kg . Only the radiation intensity in air outside the body can be readily and objectively measured in exposed individuals. The SAR is difficult to be determined for every single tissue as is different for different tissues and radiations. The best way for determining SAR is by computational approximate methods like the Finite Difference Time Domain (FDTP) method, the Finite Element Method (FEM), or the Method of Moments (MoM), (Meyer and Jacobus, 2003).

According to the ICNIRP exposure criteria, the maximum permitted radiation intensity (in mW/cm^2) for the general population exposure, is given according to radiation frequency and it is $f/2$ (f in GHz). Therefore, at 900MHz, the intensity limit according to these criteria is $0.45\text{mW}/\text{cm}^2$. At 1800 MHz the corresponding limit is $0.9 \text{ mW}/\text{cm}^2$, e.t.c). In terms of SAR the ICNIRP limits for the general population are $0.08 \text{ W}/\text{Kg}$ (for whole-body average absorbed power) and $2 \text{ W}/\text{Kg}$ (for the head and trunk). All the above values are to be averaged over any 6min period during the 24-h day. (IRPA 1988; ICNIRP 1998).

For the frequency 25-800 Hz, the IRPA-ICNIRP limits for the general population are for electric field intensity E , the value $250/f$ and for magnetic induction B , the value $50/f$, (E in kV/m , B in G , f in Hz). Therefore, at 217 Hz, (the pulse repetition frequency of digital mobile telephony radiations), the ICNIRP limits are $1.15\text{kV}/\text{m}$ and 0.23 G for up to 24h exposure during the day, (IRPA 1990; ICNIRP 1998).

As we shall see, during the years after the establishment of the IRPA-ICNIRP exposure criteria, it has been shown that the vast majority of health effects of digital mobile telephony radiations are non-thermal and a lot of biological effects were recorded at radiation intensities much lower than the values of these criteria. This is the reason why several countries in Europe have established much more stringent national exposure criteria, like Italy, Poland, Russia ($10 \mu\text{W}/\text{cm}^2$), or Salzburg (Austria), ($0.1 \mu\text{W}/\text{cm}^2$), (“EMF World Wide Standards”).

A Review of Biological, Clinical and Epidemiological Data

There is already a very large number of published studies regarding research on possible health risks from cellular mobile telephony radiations. While a large and increasing number of studies (biological, clinical and epidemiological) have recorded a variety of non-physiological changes with increased probabilities for health hazards including several types of cancer, a lot of other studies find no connection between exposure to mobile telephony radiations and health risks. Inconsistencies observed between studies are partly expected since no identical conditions can ever be attained between different studies and different labs, but also they are explained by some authors to be due to biased samples. According to a recent article in which possible secret ties between industries and University researchers are

discussed, (Hardell et al, 2007b). Since a large number of studies are funded by companies, a matter arises on how much independent these studies can be.

In the present review we shall emphasize on the studies that indicate different possible effects on living organisms, since we consider that we must take most seriously and focus the most on the possibility that is worse for living organisms and the natural environment. Additionally because of the large number of studies relating RF-microwave radiations in general, we shall concentrate on those that regard to radiations with frequencies and intensities close to those utilized by digital mobile telephony radiations (800-2450 MHz).

A. Biological Effects

Microwaves are found to produce thermally and non-thermally a large number of biological effects, in many cellular and animal studies, (Banik et al, 2003). In the case of radiations emitted by mobile telephony antennas at intensities that people are normally exposed, the effects are non-thermal as verified by different experimenters, (Diem et al, 2005; Panagopoulos et al, 2004; 2007a; 2007b; Leszczynski et al, 2002; Schirmacher et al, 2000; Velizarov et al, 1999)

Regarding non-thermal effects of RF radiations, it is a must to refer to the pioneer works of Bawin et. al. and Blackman et. al. back in the seventies and eighties although these works were relating lower frequency RF radiations. In those pioneer experiments, RF radiation with carrier frequencies 147 and 450 MHz, modulated by sinusoidal ELF signals 0-40 Hz, was found to decrease Ca^{2+} concentration in chicken brain cells. The effect was found to become maximum at modulation frequencies 6-20 Hz and at intensities 0.6-1 mW/cm², (Bawin et al 1975; 1978). Non-modulated RF signals were not found to be as bioactive as modulated ones by ELFs and additionally, these effects were found to be non-linearly depended on radiation intensity and frequency, exhibiting “windows” within which the phenomena appeared and then disappeared for values outside, (Blackman et al, 1980; 1989).

Repairable DNA damage and increased expression of heat shock protein Hsp 70 without changes in cell proliferation rates was detected in human lens epithelial cells after 2h exposure to 1.8GHz RF field, amplitude modulated at 217 Hz with 3 W/kg SAR. The DNA damage was determined by use of the comet assay, (Lixia et al, 2006).

Increased expression of genes encoding ribosomal proteins and consequently up-regulating the cellular metabolism in human cell types, was found after in vitro exposure to 900 and 1800MHz mobile phone radiation, (Remondini et al, 2006). In an other study, gene and protein expression were altered in human endothelial cell lines, after 900 MHz GSM mobile phone radiation exposure at an average SAR of 2.8 W/kg. Genes and proteins were differently affected by the exposure in each of the cell lines, suggesting that cell response to this type of radiation might be genome and proteome- dependent which in turn might explain to some extent the discrepancies in replication studies between different laboratories, (Nylund and Leszczynski, 2006).

Exposure of human endothelial cells in vitro, to GSM 900 MHz mobile phone radiation for 1h at non-thermal levels, average SAR 2 W/kg, caused transient increase in heat shock protein hsp27 phosphorylation and transient changes in protein expression levels, (Leszczynski et al, 2002).

Rapid (within minutes) induction of heat shock protein hsp70 synthesis, was found in the insect *Drosophila melanogaster*, after in vivo exposure to GSM 1900 MHz mobile phone radiation, (Weisbrot et al, 2003).

According to a theoretical report, repetitive stress caused by mobile phone radiation, leading to continuous expression of heat shock genes in exposed cells and tissues may result to cancer induction, (French et al, 2001).

Two hours of exposure by a cellular mobile phone, changed the structural and biochemical characteristics of acetylcholinesterase, an important central nervous system enzyme, resulting to a significant alteration of its activity. The enzyme was exposed within an aqueous solution at 5 cm distance from the mobile phone, (Barteri et al, 2004).

Exposure of myoglobin solution to 1.95 MHz microwave radiation for 3h at non-thermal levels was found to affect the folding of the protein and thereby changing its biochemical properties, (Mancinelli et al, 2004).

In vitro exposure for 1h of human skin fibroblasts to GSM radiation, induced alterations in cell morphology and increased the expression of mitogenic signal transduction genes, cell growth inhibitors and genes controlling apoptosis, (Pacini et al, 2002).

In an earlier study, 960 MHz GSM-like signal at SAR 0.021, 0.21 and 2.1 mW/cm² with exposure times 20, 30 and 40 min respectively, was found to decrease the proliferation rate of transformed human epithelial amnion cells. The maximum effect was reached at lower power level with a longer exposure time than at higher power level, (Kwee and Raskmark, 1998).

In another study, in vitro exposure of human peripheral blood lymphocytes to continuous 830 MHz radiation, with average SAR 1.6-8.8 W/kg, was found to produce losses and gains of chromosomes (aneuploidy), a somatic mutation leading to cancer. The effect was found to be activated via a non-thermal pathway, (Mashevich et al, 2003).

Long term exposure of rats to 900 MHz mobile phone radiation produced oxidative stress (increased oxidant products of free radicals) in retinal tissue. Melatonin and caffeic acid phenethyl ester (CAPE)- component of honeybee propolis administered daily to the animals prior to their EMR exposure, caused a significant reduction in the levels of the oxidant products, (Ozguner et al, 2006). In a previous study of the same group, melatonin was found to reverse oxidative tissue injury in rat kidneys, after 10 days exposure-30 min per day, to 900 MHz GSM radiation emitted by mobile phone, (Oktem et al, 2005).

Male mice were exposed to 1800 MHz GSM-like microwaves, 0.1 mW/cm² for two weeks on workdays, 2h per day. Then mice were anesthetized and blood samples were taken for hematology, serum chemistry and serum testosterone determinations. Additionally, testicles, epididymes, adrenals, prostates and pituitary glands were removed for histology. Red blood cell count and serum testosterone level were found to be significantly higher in the exposed groups but no significant alterations were found in the other investigated variables, (Forgacs et al, 2005).

Mice prone to the development of lymphomas, exposed for two 30 min periods per day for up to 18 months, to 900 MHz pulsed microwave radiation with a 217 Hz pulse repetition frequency at SAR ranging from 0.007 to 4.3 W/kg, developed twice the number of tumors than the unexposed ones, (Repacholi et al, 1997).

Male Wistar 35-day-old rats were exposed to 2.45 GHz radiation for 2 h/day for a period of 35 days at a power density of 0.344 mW/cm², (SAR 0.11 W/kg). After 35 days the rats were sacrificed and whole brain tissue was isolated for protein kinase C (PKC) assay. The study revealed a decrease in PKC activity. Electron microscopy study showed an increase in

the glial cell population in the exposed group. The results indicated that chronic exposures may affect brain growth and development, (Paulraj and Behari, 2006a). In another study of the same group, single strand DNA breaks were measured as tail length of comet. Fifty cells from each slide and two slides per animal were observed. The study showed that chronic exposure to microwave radiation at non-thermal levels (SAR 1 and 2 W/kg) causes statistically significant increase in DNA single strand breaks in rat brain cells, (Paulraj and Behari, 2006b).

In another study mice placed within an RF antenna park were repeatedly mated for five times while they were continuously exposed at very low levels of RF radiation (0.168-1.053 $\mu\text{W}/\text{cm}^2$). A progressive decrease in the number of newborns per maternal mouse was observed after each mating, which ended to irreversible infertility, (Magras and Xenos, 1997). In a more recent study of the same group, it was found that exposure of pregnant rats to GSM-like 940 MHz radiation at 5 $\mu\text{W}/\text{cm}^2$, resulted in aberrant expression of bone morphogenetic proteins (BMP)-(major endocrine and autocrine morphogens known to be involved in renal development), in the kidneys of newborn rats, (Pyrpasopoulou et al, 2004).

Increase in the number of micronuclei in rat bone marrow erythrocytes, a sign of genotoxicity, was observed after 30 days exposure for 2h daily, to 910 MHz microwave radiation, (Demsia et al, 2004).

In several other mammal studies, no effects were found, in regards to genotoxicity of second generation mobile telephony (GSM, DCS) and third generation, "universal mobile telecommunication system" (UTMS) radiations, (Sommer et al 2007; Oberto et al 2007; Juutilainen et al 2007; Tillmann et al 2007; Gatta et al 2003).

The mortality of chicken embryos was found to increase to 75% from 16% in the control group, after exposure to radiation from a GSM mobile phone, (Grigor'ev, 2003). This result is in agreement with the increased mortality of fertilised chicken eggs that was recorded after irradiation by low power 9.152 GHz pulsed and continuous-wave microwaves, (Xenos and Magras, 2003).

Several studies have reported that microwave exposures increase the permeability of the blood-brain barrier (BBB), an hydrophobic barrier made by endothelial cells to protect the mammalian brain from harmful compounds in the blood. A Swedish group has reported that 915 MHz microwaves at non-thermal intensities causes leakage of albumin into the brain through the BBB in rats, accumulating in the neurons and glial cells which surround the capillaries in the brain, (Salford et al, 1994). The same group reported that GSM mobile phone radiation from a test mobile phone with a programmable constant power output, opens the BBB for albumin, resulting to damage of brain cells in rats. The power density and SAR were within the ICNIRP limits, (Salford et al 2003). These were the first experiments that indicated cell damage caused by mobile phone radiation although this radiation was not a real mobile phone signal. However in an earlier study of the same group, continuous-wave and pulsed 915 MHz radiation at relatively high intensities, 1 W and 2 W respectively, was not found to damage brain or promote brain tumour development in rats, (Salford et al. 1993).

Exposure of an in vitro BBB model, consisted by rat brain cells growing in a culture with pig blood cells, exposed to 1800 MHz microwave radiation pulsed at 217 Hz repetition rate (DCS-like), at SAR 0.3-0.46 W/kg, increased the permeability to sucrose of the BBB twice compared to the control culture. No significant temperature rise was detected during the exposures, (Schirmacher et al, 2000). In a latter study of the same group, in vitro exposure of

three other BBB models with distinctly higher barrier tightness than the previously used one, did not cause any effect on the permeability of the BBB of the models, (Franke et al, 2005).

In regards to DNA damage or cell death induction due to microwave exposure, in a series of early experiments, rats were exposed to pulsed and continuous-wave 2450 MHz radiation for two hours at an average power density of 2 mW/cm² and their brain cells were subsequently examined for DNA breaks by “comet” assay. The authors found a dose-dependent (0.6 and 1.2 W/kg whole body SAR) increase in DNA single-strand and double-strand breaks, four hours after the exposure to either the pulsed or the continuous-wave radiation, (Lai and Singh 1995; 1996). The same authors found that melatonin and PBN (N-tert-butyl-alpha-phenylnitron) both known free radical scavengers, block the above effect of DNA damage by the microwave radiation, (Lai and Singh 1997). Although these experiments were the first to report DNA damage by microwaves, the radiation intensity (2mW/cm²) was relatively high, exceeding the international exposure limits (ICNIRP 1998) and additionally the radiation frequency was the same as in microwave ovens. This is why the authors of this review cannot be sure on whether the reported effects were thermal or non-thermal.

In vitro exposure of mouse fibroblasts and human glioblastoma cells to 2450 MHz, (Malyapa et al, 1997a), 835.62 MHz and 847.74 MHz (Malyapa et al, 1997b), radiations at SAR 0.6 W/kg, was not reported to damage DNA as measured by comet assay.

A number of recent studies have reported DNA damage, or cell damage, or cell death, induced by mobile telephony or similar RF radiations at non-thermal intensity levels, (Aitken et al, 2005; Diem et al 2005; Panagopoulos et al 2007; Salford et al, 2003; Markova et al, 2005; Caraglia et al, 2005; Nikolova et al, 2005), while some other studies did not find any such connection, (Hook et al, 2004; Capri et al, 2004a; 2004b; Meltz 2003; Cranfield et al, 2003). Aitken et al 2005, reported damage to mitochondrial genome and the nuclear beta-globin locus in the spermatozoa of mice exposed to 900 MHz, 0.09 W/kg SAR, for 7 days, 12h per day. Diem et al 2005, reported single and double-strand DNA breakage in cultured human and rat cells exposed to 1800 MHz mobile phone-like radiation. Panagopoulos et al 2007a, found DNA fragmentation at a very high degree, caused in the reproductive cells of female *Drosophila* insects only by few min daily exposure to a real mobile phone signal for only few days. These were the first experiments that showed extensive DNA damage and cell death by real digital mobile phone GSM and DCS signals. Previous experiments of the same group had shown a large decrease in the reproductive capacity of the same insect, caused by real mobile phone similar exposures, (Panagopoulos et al, 2004).

B. Clinical Studies on Humans. Effects on EEG, EDA, Melatonin, etc

Mobile telephony radiation is found in several studies to affect electroencephalograms (EEG), electrodermal activity (EDA) and the synthesis rate of hormones like melatonin, in humans.

In a series of early experiments performed by a Finish group, GSM mobile phone exposure was found to alter the EEG oscillatory activity of healthy adult subjects, in the 6-8 and 8-10 Hz frequency bands during cognitive (visual memory) tasks, (Krause et al, 2000). In more recent experiments of the same group, exposure of 10-14 year old children to mobile phone GSM field while performing an auditory memory task, induced changes in their brain oscillatory EEG responses in the frequencies 4-8 Hz and 15 Hz, (Krause et al, 2006).

Exposure for 30 min to pulse modulated 900 MHz mobile phones-like EMF, increased waking regional cerebral blood flow (rCBF) and enhanced EEG power in the alpha frequency range (8-12 Hz) prior to sleep onset and during sleep. Exposure to the same field without pulse modulation did not enhance power in waking or sleep EEG, (Huber et al, 2002). In another set of experiments of the same group, 30 min exposure to the same 900 MHz GSM-like field during waking period preceding sleep, increased the spectral power of the EEG in non-rapid eye movement sleep. The maximum increase occurred in the 9.75-11.25 Hz and 12.5-13.25 Hz frequency ranges during the initial part of the sleep. Since exposure during waking, modified the EEG during subsequent sleep, the changes in the brain function induced by mobile telephony radiation are considered to outlast the exposure period, (Huber et al, 2000).

Mobile phone exposure prior to sleep was found to decrease rapid eye movement sleep latency and to increase EEG spectral power in the 11.5-12.5 Hz frequency, during the initial part of sleep following exposure, (Loughran et al, 2005).

Some other studies have failed to find any effects of mobile phone-microwave exposures on EEG during cognitive testing, or to replicate earlier findings, (Röschke and Mann, 1997; Wagner et al., 1998).

Mobile phone radiation was found to affect the evoked neuronal activity of the central nervous system (CNN) as represented by EDA, an index of the sympathetic nervous system. Mobile phone exposure was found to lengthen the latency of EDA (Skin Resistance Response), irrespectively of the head side next to mobile phone, (Esen and Esen, 2006). Therefore, mobile phone exposure may increase the response time of users with different negative consequences, like for example the increase in the risk of phone-related driving hazards, e.t.c.

A statistically significant increase of chromosomal damage was found in blood lymphocytes of people who used GSM 900 MHz mobile phones, compared to a control group of non-users, matched according to age, sex, health status, drinking and smoking habits, working habits, and professional careers. The increase was even greater for users who were smoker-alcoholic, (Gadhia et al, 2003)

In another type of clinical study, exposures of humans to GSM 900 MHz and DCS 1800 MHz mobile phones fields for 35 min, were not found to change significantly arterial blood pressure or heart rate during or after the exposure, (Tahvanainen et al, 2004).

Prolonged use of mobile phone, (more than 25 min per day), was found to induce a reduction in melatonin production among male users. The effect was enhanced by additional exposure to 60 Hz ELF magnetic field, (Burch et al, 2002).

Two studies about possible immediate- short term effects of GSM and UTMS (third generation of mobile networks)-like exposure on well being and cognitive performance in humans based on questionnaires, found contradictive results. The first (Zwamborn et al, 2003) reported no effects of GSM-like exposure, while the UTMS-like exposure was found to reduce well-being and cognitive performance. The second, (Regel et al, 2006) reported no effects at all from either type of radiation. The opinion of the authors of this review is that studies based on questionnaires cannot be as much objective as studies based on measurable indexes like EEG or EDA. Besides, it would be unlikely that subjects would report themselves immediate effects on their well-being.

C. Epidemiological Studies

According to the Swedish Prof. L. Hardell and his research group, the concluding results of up to date epidemiological studies among users for more than ten years use of mobile phones indicate consistently an increased risk for acoustic neuroma and glioma, especially for ipsilateral exposure, (Hardell et al, 2007a). Earlier work of the same research group had found a connection between digital (2nd generation) and analogue (1st generation) mobile phones use and malignant brain tumors, highest for more than ten years latency period, (Hardell et al, 2006).

Another review study of the Austrian Prof. M.Kundi conducted few years ago, states as the resume from several epidemiological and experimental studies, that long term exposure to mobile phone emissions (analogue and digital) constitutes a small to moderate increased risk for developing certain types of cancer, (Kundi, 2004).

Several other studies had not found any association between mobile phone use and cancer, (Inskip et al, 2001; Johansen et al, 2001; Muscat et al, 2002).

A major difficulty in epidemiological studies among mobile phone users is the variation of parameters governing the exposure from hand held mobile phones, i.e. the distance from the nearest base station which can considerably change the intensity of the radiation emitted by the phone, the actual duration of daily use, e.t.c. Nevertheless, the studies done on habitants living close to base stations are more consistent since the station emits a more constant radiation level on a daily basis and therefore a person residing nearby, receives a measurable radiation at least for several hours per day.

A recent Egyptian study (Abdel-Rassoul et al, 2007) found that inhabitants living nearby mobile telephony base stations may develop a number of neuropsychiatric problems like headaches, memory changes, dizziness, tremors, depression, sleep disturbances, reported also in previous studies as “microwave syndrome” (Navarro et al 2003), plus changes in the performance of neurobehavioral functions. Similar results were found by other studies in different countries like in France, (Santini et al 2003), Poland (Bortkiewicz et al 2004), Spain (Navarro et al 2003), Austria (Hutter et al 2006).

Other epidemiological studies have reported diminishes in the populations of birds around mobile telephony base stations at distances 100-600m from the masts in Belgium, (Everaert and Bauwens 2007) and within 200m from the masts in Spain (Balmori 2005). These studies are in agreement with earlier biological studies which had reported increased mortality of avian embryos, exposed to low levels (5-120 $\mu\text{W}/\text{cm}^2$) of RF antennae radiation, (Xenos and Magras, 2003).

The Design of Bioelectromagnetic Experiments and a Reason for Inconsistencies

As described in the previous paragraphs, there are frequently contradictory results in the bioelectromagnetic experiments performed by different labs. One factor that we have found to be very important and able to completely change the results of a biological experiment is the influence of the stray electromagnetic fields that exist inside any lab.

Within a usual room inside a house or laboratory there are 50-60 Hz fields due to the electric wirings and electrical appliances. Close to the walls, near to sockets or close to electrical appliances one can measure electric fields up to 50 V/m and magnetic fields up to 10 mG. Such fields are found to affect biomolecules, cells and whole organisms in different ways and therefore to affect the outcome of any biological experiment, (Goodman E. et al. 1995; Panagopoulos et al. 2002; Weaver and Astumian 1990). Prior to the design of any biological experiment, a careful scanning of stray fields inside the lab is necessary. The experiments should be performed at the place with the minimum stray fields and special care should be taken in having the control under identical conditions with the exposed groups except only for the factor studied. Temperature, light and humidity are additional important factors that should be identical between exposed and control groups.

Before the relatively recent evolution of knowledge in the field of Bioelectromagnetism, ambient electromagnetic fields within the labs were not taken into account in biological experiments. But living organisms are very sensitive to external electromagnetic fields, natural or artificial ones. Rooms or devices used as incubators, are constructed to keep a constant temperature, humidity, e.t.c. in their internal space, but usually are sources of EMFs from their own electrical circuits. A specialized physicist should always be member of any experimental team for taking good care of such factors.

Effects of Mobile Telephony Radiation on a Model Organism

Introduction

In order to study the ability of the electromagnetic signals emitted by cellular mobile telephony antennas to affect the biological function of living organisms, we used a biological model, the reproductive capacity of the insect *Drosophila melanogaster*, a well studied experimental animal with many advantages, including its short life cycle and the good timing of its metamorphic stages and developmental processes, (King 1970). Especially the good timing of this insect's early developmental stages (oogenesis, spermatogenesis, embryogenesis, larval and pupal stages), under certain environmental conditions (i.e. temperature, humidity, food e.t.c.), is a very important feature, on which our experimental protocols were based.

In order to study the effects of mobile telephony radiation on the reproductive capacity, we exposed the insects to real mobile phone signals, emitted by commercially available handsets.

The basic cellular processes are identical in insect and mammalian cells. In addition, insects (particularly *Drosophila*) are much more resistant, at least to ionizing electromagnetic radiation, than mammals, (Koval and Kazmar 1988, Koval et al 1979, 1977, Abrahamson et al 1973). Therefore, a proper experimental protocol relating *Drosophila* can be very useful in assessing the bioactivity of electromagnetic radiation in general, (including non-ionizing radiation and electromagnetic fields).

Our experiments, regarding few minutes daily exposure of this model organism for only few days, to cellular mobile phone signals, have shown a large decrease in the reproductive capacity, affecting both sexes (Panagopoulos et al 2004). Both systems of digital mobile telephony radiation used in Europe, GSM 900 MHz and DCS 1800 MHz were found to

decrease the insect's reproductive capacity, but GSM 900 MHz was found to be even more bioactive than DCS 1800 MHz, mainly due to the higher intensity of GSM 900 MHz antennas compared to DCS 1800 MHz ones, (Panagopoulos et al 2007b; 2007a). The decrease in the reproductive capacity was found to be due to induced cell death (DNA fragmentation) in the gonads, caused by both types of mobile telephony signals, (Panagopoulos et al 2007a). Unpublished experiments of ours presented here for the first time, show that the bioactivity is strongly and non-linearly dependent on the intensity of the radiation, becoming maximum for intensities higher than $200 \mu\text{W}/\text{cm}^2$ and within an "intensity window" around $10 \mu\text{W}/\text{cm}^2$.

Materials and Methods

Experimental Animal

We used *Drosophila melanogaster* flies, wild-type strain, Oregon R, held in glass bottles with standard food, kept in incubator at 25°C , with 12-h periods of light and darkness and 70% relative humidity, cultured according to standard methods, (Panagopoulos et al 2004).

The food consisted of 450ml water, 4g agar, 13g yeast, 32g rice flour, 16g sugar, 25g tomato pulp. The mixture was boiled for over 10min to ensure sterility, which was preserved by the addition of 2ml propionic acid and 2ml ethanol. This food quantity was enough for 25-30 glass vials which were sterilized before the food was added.

In each experiment, we collected newly emerged adult flies from the stock early in the afternoon, anesthetized them lightly with diethyl ether and separated males from females. We divided the collected flies in groups of ten in standard laboratory cylindrical glass vials, with 2.5cm diameter and 10cm height, with standard food, which formed a smooth plane surface, 1cm thick at the bottom of the vials. The vials were closed with cotton plugs.

Exposure System

Before each set of experiments we measured the mean power density of the radiation emitted by the mobile phone handset in the RF range at 900MHz and/or 1800MHz, with the field-meter, "RF Radiation Survey Meter, NARDA 8718", with its probe inside a glass vial similar to the ones we used for the insects in our experiments. In addition, we measured in the same way the mean electric and magnetic field intensities at the Extremely Low Frequency (ELF) range, with the field-meter, "Holaday HI-3604, ELF Survey Meter".

The experimenter's position in relation to the mobile phone during the measurements was the same as during the exposures. The mobile phone was held close to the experimenter's head with its antenna facing downward. The exposures and the field measurements, took place in a quiet but not sound-isolated room to simulate the actual conditions to which a user is subjected during a normal conversation on the mobile phone. The room conditions and the positions of all items around the experimental bench were always the same. Exposures and measurements of mobile phone emissions were always conducted at the same place where the mobile phone had full perception of both GSM and DCS signals. The handset was fully charged before each set of exposures or measurements.

In the most new digital cell phone handsets, the antenna is in the back and upper side of the device. This can be easily verified by measuring the emitted radiation holding the probe of the field meter in contact with different parts of the handset's surface.

The measured exposure values were in general within the established exposure limits, (ICNIRP 1998).

We used commercially available digital mobile phone handsets in all the sets of our experiments, in order to analyze effects of real mobile telephony exposure conditions. As far as we know, we were the first to use a commercially available mobile phone handset itself in biological experiments, (Panagopoulos et al 2000a). The obvious reason was that these devices are the most powerful RF transmitters in our immediate daily environment. Thus, instead of using simulations of digital mobile telephony signals with constant parameters (frequency, intensity etc), or even “test mobile phones” programmed to emit mobile telephony signals with controllable power or frequency, we used real GSM, DCS signals which are never constant, since there are continuous changes in their intensity and frequency. Electromagnetic fields with changing parameters are found to be more bioactive than fields with constant parameters, (Goodman E.M. et al 1995; Diem et al 2005), probably because it is more difficult for living organisms to get adapted to them. Experiments with constant GSM or DCS signals can be performed, but they do not simulate actual conditions. Later other experimenters also started to use mobile phone handsets as exposure devices apparently for the same reasons, (Weisbrot et al 2003; Barteri et al 2005).

We exposed the flies within the glass vials by placing the antenna of the mobile phone outside of the vials, in contact with or at different distances from the glass wall and parallel to the vial’s axis. The total duration of exposure was 6min per day in one dose and we started the exposures on the first day of each experiment (day of eclosion). The exposures took place for a total of 2 to 6 days in each experiment depending on the kind of the experiment, as described below. The daily exposure duration of 6min, was chosen in order to have exposure conditions that can be compared with the established exposure criteria, (ICNIRP 1998). Besides, early experiments had shown that only few minutes of daily exposure were enough to produce a significant effect on the insect’s reproductive capacity (Panagopoulos et al, 2000a).

The experimenter could speak on the mobile phone during connection (this we called, “modulated” or “speaking” emission), or could just stay silent, (“non-modulated” or “non-speaking” emission, or DTX mode). The intensity of the emitted radiation increases about ten times when the user speaks during connection, than when there is no speaking, (Panagopoulos et al, 2000a).

Exposure Procedures

We carried out six sets of experiments: In the first set, we exposed the insects to the mobile phone’s GSM 900 MHz field while the mobile phone was operating in non-speaking mode, (non-modulated emission or DTX). In the second set of experiments, the mobile phone was operating in speaking mode, (modulated emission) during the exposures. In the third set of experiments we investigated the effect of the mobile phone signal on the reproductive capacity of each sex separately. In the fourth set of experiments we compared the bioactivity between GSM 900 MHz and DCS 1800 MHz types of mobile telephony signals. In the fifth set of experiments we exposed the insects to different distances (intensities), from the mobile phone antenna from 0 to 100 cm, for both types of radiation. Finally, in the sixth set of experiments we tested the ability of GSM and DCS fields to induce DNA fragmentation (cell death) in the ovarian cells of the female insects during oogenesis.

In every single experiment we separated the newly emerged collected adult flies to exposed (E) and sham-exposed (SE)/control (C) groups. Each one of the groups consisted

always of ten female and ten male, newly emerged flies. The sham exposed groups had identical treatment as the exposed ones, except that the mobile phone during the 6-min “exposures”, was turned off.

Every time before each exposure, the cotton plugs were pushed down in the glass vials in order to confine the flies to a small area of about 1cm height between the cotton and the food so as to provide roughly even exposure to all flies. After the exposure, the cotton plugs were pulled back to the top of the vials, and the vials were put back in the culture room.

In every group of insects in all the sets of experiments, we kept the ten males and the ten females for the first 48h of the experiment in separate glass tubes. At eclosion, adult female flies have already in their ovaries eggs at the first pre-yolk stages and oogenesis has already started. The eggs develop through 14 distinct stages, until they are ready to be fertilized and laid, and the whole process of oogenesis lasts about 48h. By the end of the second day of their adult life, the female flies have in their ovipositors the first fully developed egg chambers of stage 14th, ready to be fertilized and laid, (King 1970; Panagopoulos et al 2004). At the same time, the first mature spermatozoa, (about 6h after eclosion) and the necessary paragonial substances (about 12h after eclosion) in male flies have already been developed (King 1970; Stromnaes and Kvelland 1962; Connolly and Tully 1998). Keeping males separately from females for the first 48h of the experiment ensures that the flies are in complete sexual maturity and ready for immediate mating and laying of fertilized eggs.

After the first 48h of each experiment, the flies were anesthetized very lightly again and males and females of each group were put together (ten pairs) in another glass tube with fresh food, allowed to mate and lay eggs for 72h. During these three days, the daily egg production of *Drosophila* is at its maximum (from the 3rd to 5th day of its adult life), then stays at a plateau or declines slightly for the next 5 days and diminishes considerably after the 10th day of adult life (Bos and Boerema 1981; Shorrocks 1972; Ramirez et al 1983).

On the sixth day of each experiment in all six sets of experiments, the flies were removed from the glass vials and the vials were maintained in the culture room for six additional days, without further exposure.

After the last six days, most F₁ embryos (deriving from the laid eggs) are in the stage of pupation, where they can be clearly seen with bare eyes and easily counted on the walls of the glass tubes, as at the last stages before pupation, the larvae leave the food, crawling up the walls of the glass vials. There may be a few embryos still in the last stages as larvae, which are big enough and ready for pupation (on the surface or already away from the food), so that they can be easily counted. [If the remaining larvae are still many and the counting is imprecise, the experimenter can wait an additional day and recount the pupae]. There may be also already a few newly emerged F₁ adult flies, which can also be counted easily.

During the last six days, we inspected the surface of the food within the glass vials under the stereo-microscope for any non-developed laid eggs or dead larvae, something that we did not see in our experiments (empty egg-shells can be seen after hatching). The number of observed exceptions (non-developed eggs or dead larvae), both in exposed and control groups (less than 5%) was within the Standard Deviation of progeny number. [The insignificant percentage of F₁ egg and larvae mortality is due to the fact that the paternal-maternal flies were newly emerged during the first 2-5 days of their adult lives]. Therefore the number of pupae in our experiments corresponded to the number of laid eggs (oviposition). Furthermore, the counting of pupae can be done without any error at all, whereas the counting of laid eggs under a stereo-microscope is subject to considerable error.

The oviposition of *Drosophila* is influenced by many factors, like temperature, humidity, prior anesthesia, crowding, food, (King 1970). Special care must be taken to keep all these factors constant. Experience in handling the flies is necessary to prevent accidental deaths.

This number of F₁ pupae under the above described conditions, during the insect's three days of highest oviposition, is that we have defined as the Insect's Reproductive Capacity and this is the biological index we have used to examine the bioactivity of electromagnetic radiation-field.

The temperature during the exposures was monitored within the vials with a mercury thermometer with an accuracy of 0.05°C.

In the sixth set of experiments, after the additional last exposure in the morning of the sixth day from the beginning of each experiment, the flies were removed from the glass vials, and the ovaries of females were dissected into individual ovarioles and fixed for TUNEL assay. The vials were then maintained in the culture room for six additional days, without further exposure, in order to count the F₁ pupae as in all the sets of experiments.

TUNEL Assay

A widely used method for identifying cell death is TUNEL assay. By use of this method, fluorescein dUTP is bound through the action of terminal transferase, onto fragmented genomic DNA which then becomes labelled by characteristic fluorescence. The label incorporated at the damaged sites of DNA is visualized by fluorescence microscopy, (Gavrieli et al, 1992).

Each *Drosophila* ovary consists of 16 to 20 ovarioles. Each ovariole is an individual egg assembly line, with new egg chambers in the anterior moving toward the posterior as they develop, through the 14 successive stages as described, until the mature egg reaches the oviduct.

To determine the ability of GSM and DCS radiation to act as possible stress factors able to induce cell death during early and mid oogenesis, we used TUNEL assay, as follows: Ovaries were dissected in Ringer's solution and separated into individual ovarioles from which we took away egg chambers of stages 11-14. In egg chambers of stages 11-14 programmed cell death takes place normally in the nurse cells and follicle cells. Thereby we kept and treated ovarioles and individual egg chambers from germarium up to stage 10. Samples were fixed in PBS solution containing 4% formaldehyde plus 0.1% Triton X-100 (Sigma Chemical Co., Germany) for 30min and then rinsed three times and washed twice in PBS for 5 min each. Then samples were incubated with PBS containing 20 µg/ml proteinase K for 10 minutes and washed three times in PBS for 5 min each. In situ detection of fragmented genomic DNA was performed with Boehringer Mannheim kit containing fluorescein dUTP for 3h at 37°C in the dark. Samples were then washed six times in PBS for 1h and 30 min in the dark and finally mounted in antifading mounting medium (90% glycerol containing 1,4-diazabicyclo (2.2.2) octane (Sigma Chemical Co., Germany) to prevent from fading and viewed under a Nikon Eclipse TE 2000-S fluorescence microscope.

Results and Discussion

In the first two sets of experiments, we separated the insects into two groups: a) the Exposed group (E) and b) the Sham Exposed group (SE). The 6-min daily exposures took place for the first five days of each experiment.

In the first three sets of experiments, the exposures were performed by GSM 900 MHz mobile phone radiation-field. Before the exposures, we measured radiation and field intensities, as described above. In the RF range, the measured mean power density for 6min of modulated emission (M), with the antenna of the mobile phone outside of the glass vial in contact with the glass wall and parallel to the vial's axis was 0.436 ± 0.060 mW/cm². The non-modulated (NM) corresponding measured mean value, was 0.041 ± 0.006 mW/cm². In the ELF range, the measured values for modulated field, excluding the ambient electric and magnetic fields of 50Hz, were 6.05 ± 1.62 V/m electric field intensity and 0.10 ± 0.06 mG magnetic field intensity. The corresponding non-modulated values were 3.18 ± 1.10 V/m and 0.030 ± 0.003 mG. All given values are average from eight separate measurements of each kind \pm Standard Deviation (SD). These values are typical for all commonly used GSM 900 MHz mobile phone handsets.

1. Effect of Non-Modulated GSM radiation-field on the Reproductive Capacity

We carried out four experiments (1.1-1.4) with non-modulated field, (non-speaking emission). The exposure parameters in this case simulate the situation when a user listens through the mobile phone during connection.

Results are listed in Table 1.

Table 1 shows the mean number of F₁ pupae (corresponding to the number of laid eggs) per maternal fly in the groups E(NM) exposed to Non-Modulated (NM), GSM 900 MHz mobile phone field and in the corresponding sham exposed (control) groups SE(NM) during the first three days of the insect's maximum oviposition.

The Non-Modulated GSM 900 MHz signals, decreased the insect's reproductive capacity by up to 20% in relation to the unexposed groups with six min daily exposure for five days. No temperature increases were detected within the vials during the exposures.

Table 1. Effect of Non-Modulated GSM field on the Reproductive Capacity of *Drosophila melanogaster*

Experiment No	Groups	Mean Number of F ₁ Pupae per Maternal Fly	Deviation from Control
1.1	E(NM)	9.7	-16.38%
	SE(NM)	11.6	
1.2	E(NM)	10	-15.96%
	SE(NM)	11.9	
1.3	E(NM)	9.8	-20.16%
	SE(NM)	12.4	
1.4	E(NM)	10.4	-19.38%
	SE(NM)	12.9	
Average \pm SD	E(NM)	9.975 ± 0.31	-18.24%
	SE(NM)	12.2 ± 0.57	

Statistical analysis, (single factor ANOVA test) shows that the probability that mean oviposition differs between the exposed and the sham exposed groups, owing to random

variations, is $P < 5 \times 10^{-4}$. Therefore, the decrease in the reproductive capacity is due to the effect of the GSM field.

2. Effect of Modulated GSM Radiation-field on the Reproductive Capacity

We carried out four experiments (2.1-2.4), with modulated emission (the experimenter was speaking close to the mobile phone's microphone, during the exposures). The exposure parameters in this case simulate the situation when a user speaks on the mobile phone during connection. Results are listed in Table 2.

Table 2 shows the mean number of F_1 pupae (corresponding to the number of laid eggs) per maternal fly in the groups E, exposed to "Modulated" GSM field and in the corresponding sham exposed groups, SE, during the first three days of the insect's maximum oviposition.

The Modulated GSM 900 MHz signals induced a large decrease in the insect's reproductive capacity up to 60% as compared to the unexposed groups. No temperature increases were detected during the exposures and thus these effects are considered as non-thermal.

Table 2. Effect of Modulated GSM field on the Reproductive Capacity of *Drosophila melanogaster*

Experiment No	Groups	Mean Number of F_1 Pupae per Maternal Fly	Deviation from Control
2.1	E(M)	6.7	-48.85%
	SE (M) (Control)	13.1	
2.2	E	5.1	-56.78%
	SE (M) (Control)	11.8	
2.3	E	5.6	-53.72%
	SE (M) (Control)	12.1	
2.4	E	6	-53.125%
	SE (M) (Control)	12.8	
Average \pm SD	E (M)	5.85 ± 0.67	-53.01%
	SE (M) (Control)	12.45 ± 0.6	

The reproductive capacity was much more decreased by modulated emission, (50-60%), than by non-modulated emission, (15-20%). Thus the effect is strongly dependent on radiation-field intensity. At the same time, the intensity of the modulated signal, is about ten times more powerful than the non-modulated signal. Thereby, the effect is not linearly dependent on radiation intensity.

The results from the first two sets of experiments are represented, in Figure 1.

The statistical analysis shows that the probability that mean oviposition differs between the exposed and the sham exposed groups, owing to random variations, is very small, $P < 10^{-5}$. Thus the recorded effect is due to the GSM signal.

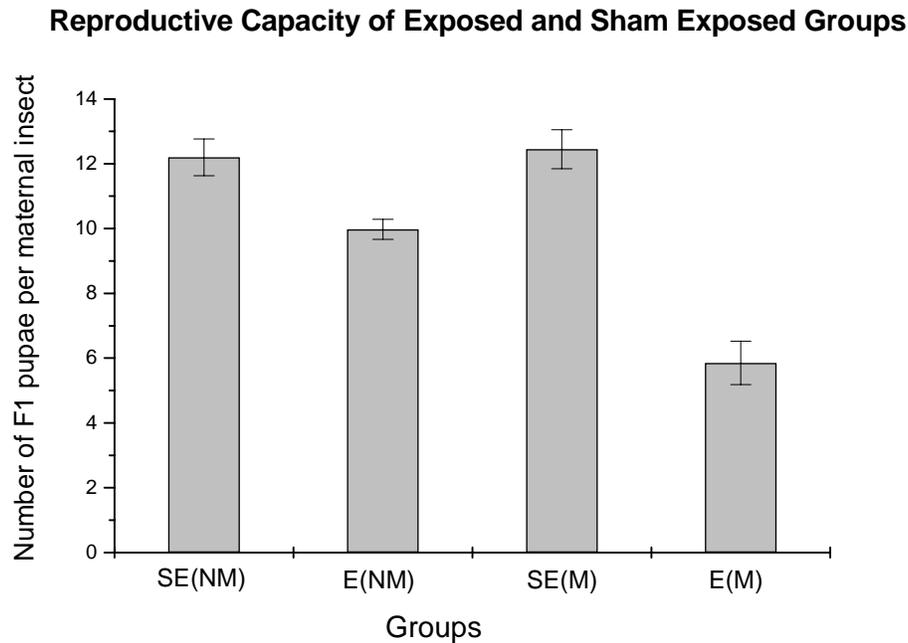


Figure 1. Reproductive Capacity of the groups exposed to non-modulated and modulated GSM 900 MHz field [E(NM), E(M)] and the corresponding sham exposed, [SE(NM), SE(M)], groups. [The error bars correspond to Standard Deviation].

3. Effects on the Reproductive Capacity of Each Sex

A third set of experiments (C) was carried out in order to record the effect of the GSM 900 MHz field on the reproductive capacity of each sex separately. The mobile phone was operating in speaking mode during the 6 min exposures, and the insects were separated into four groups (each one consisting again 10 male and 10 female insects): In the first group (E1), both male and female insects were exposed. In the second group (E2), only the females were exposed. In the third group (E3), we exposed only the males and the fourth group (SE) was sham exposed (control). Therefore in this third set of experiments, the 6-min daily exposures took place only during the first two days of each experiment while the males and females of each group were separated and the total number of exposures in each experiment was 2 instead of 5.

The results from this set of experiments are listed in Table 3 and represented graphically in Figure 2.

The results of this set of experiments show that the GSM field affects the reproductive capacity of both female and male insects. The female insects (E2) were more affected than males (E3) in these experiments. This is expected to be due to the fact that, by the time we started the exposures, spermatogenesis was already almost completed in male flies, while oogenesis had just started, (King 1970; Panagopoulos et al 2004).

Statistical analysis (single factor ANOVA test) shows that the probability that mean oviposition differs between the four groups because of random variations is $P < 10^{-7}$.

Table 3. Effect of “Modulated” GSM field on the Reproductive Capacity of each sex

Experiment No	Groups	Mean Number of F ₁ Pupae Per Maternal Fly	Deviation from Control
3.1	SE(Control)	13.2	
	E1	8.5	-35.61%
	E2	9.4	-28.79%
	E3	11.7	-11.36%
3.2	SE (Control)	13.8	
	E1	7.6	-44.93%
	E2	8.9	-35.51%
	E3	12.1	-12.32%
3.3	SE (Control)	12.9	
	E1	7.8	-39.53%
	E2	9.3	-27.91%
	E3	11	-14.73%
3.4	SE (Control)	13.5	
	E1	6.9	-48.89%
	E2	7.8	-42.22%
	E3	12.2	-9.63%
Average \pm SD	SE (Control)	13.35 \pm 0.39	
	E1	7.7 \pm 0.66	-42.32%
	E2	8.85 \pm 0.73	-33.71%
	E3	11.75 \pm 0.54	-11.985%

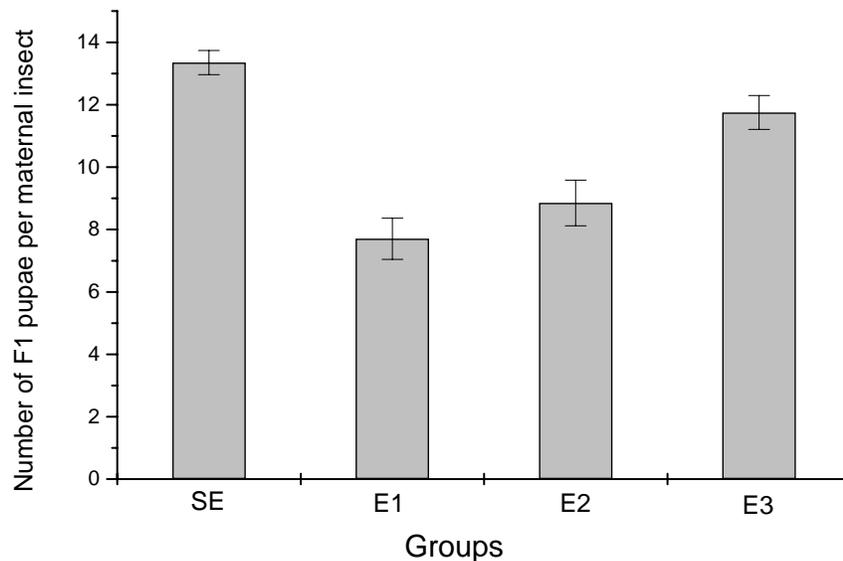
Effect of GSM field on the Reproductive Capacity of each sex

Figure 2. Effect of Modulated GSM field on the reproductive capacity of each sex of *Drosophila melanogaster*. Average mean number of F₁ pupae \pm SD per maternal insect. SE: sham exposed groups, E1: groups that both sexes were exposed, E2: groups in which only the females were exposed, E3: groups in which only the males were exposed.

In the following fourth, fifth and sixth set of experiments, we used a dual band cellular mobile phone that could be connected to either GSM 900 or DCS 1800 networks simply by changing SIM (“Subscriber Identity Module”) cards on the same handset. The highest Specific Absorption Rate (SAR), given by the manufacturer for human head, was 0.89 W/Kg. The exposure procedure was the same. The experimenter spoke on the mobile phone’s microphone during the exposures. The GSM and DCS fields were thus “modulated” by the human voice, (“speaking emissions” or “GSM basic”).

4. Comparison of Bioactivity between GSM 900 MHz and DCS 1800 MHz

In this set of experiments we separated the insects into four groups: a) the group Exposed to GSM 900MHz field with the mobile phone antenna in contact with the glass vial containing the flies (named as “900”), b) the group exposed to GSM 900MHz field with the antenna of the mobile phone at 1cm distance from the vial (named as “900A”), c) the group exposed to DCS 1800MHz field with the mobile phone antenna in contact with the glass vial (named as “1800”), and d) the Sham Exposed (Control) group (named as “SE”). The comparison between first and third group represents comparison with the usual exposure conditions between GSM 900 and DCS 1800 users, while comparison between second and third group represents comparison between possible effects of the RF frequencies of the two systems under equal radiation intensities. Therefore the second group (900A) was introduced for better comparison of effects between the two types of radiation.

Measured mean power densities in contact with the mobile phone antenna for six min of modulated emission, were 0.407 ± 0.061 mW/cm² for GSM 900 MHz and 0.283 ± 0.043 mW/cm² for DCS 1800 MHz. As was expected GSM 900 MHz intensity at the same distance from the antenna and with the same handset was higher than the corresponding DCS 1800 MHz. For the better comparison between the two systems of radiation we measured the GSM power density at different distances from the antenna and found that at 1cm distance, the GSM 900 MHz intensity was 0.286 ± 0.050 mW/cm², almost equal to DCS 1800 MHz at zero distance. Measured electric and magnetic field intensities in the ELF range for modulated field, excluding the ambient electric and magnetic fields of 50Hz, were 22.3 ± 2.2 V/m electric field intensity and 0.50 ± 0.08 mG magnetic field intensity for GSM at zero distance, 13.9 ± 1.6 V/m, 0.40 ± 0.07 mG correspondingly for GSM at 1 cm distance and 14.2 ± 1.7 V/m, 0.38 ± 0.07 mG correspondingly for DCS at zero distance. All these values are averaged over ten separate measurements of each kind \pm standard deviation (SD).

Except for the power density - field measurements of the mobile phone emissions, we obtained the spectra of both types of radiation, plus the background spectrum in our lab, (Fig. 3). Each one of the two types of radiation gave a unique frequency spectrum. While GSM 900MHz gives a single peak around 900MHz, (Fig. 3b), DCS 1800MHz gives a main peak around 1800MHz and a smaller one around 900MHz, (Fig. 3c). The spectra were obtained by a Hewlett Packard 8595 E, (9 kHz-6.5 GHz), spectrum analyzer (USA).

We carried out ten replicate experiments. Results are listed in Table 4 and represented graphically, in Figure 4.

The results from this set of experiments show that the reproductive capacity in all the exposed groups is significantly decreased compared to the sham exposed groups. The decrease is maximum in the 900 groups, (48.25% compared to SE) and smaller in the 900A and the 1800 groups, (32.75% and 31.08% respectively), (Table 4). Although the decrease was even smaller in the 1800 groups than in 900A, differences between the 900A and 1800 groups were found to be within the standard deviation, (Table 4, Figure 4).

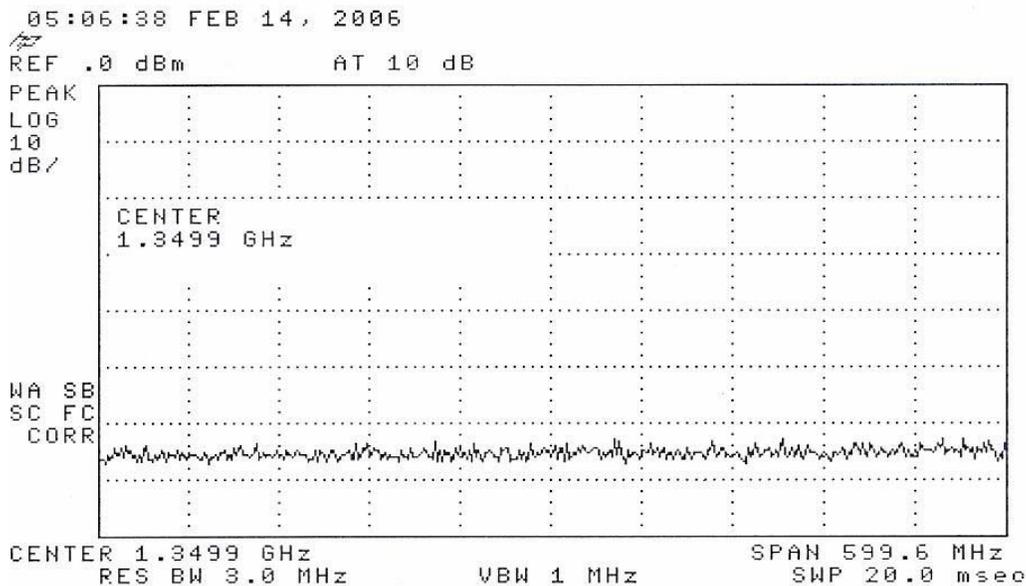
The statistical analysis shows that the probability that the reproductive capacity differs between groups, owing to random variations, is negligible, $P < 10^{-18}$.

Again, we did not detect any temperature increases, within the glass vials during the exposures.

The differences in the reproductive capacity between the groups were greater between 900 and 900A (owing to intensity differences between the two types of radiation) and much smaller between 900A and 1800, (owing to frequency differences between GSM and DCS), (Table 4).

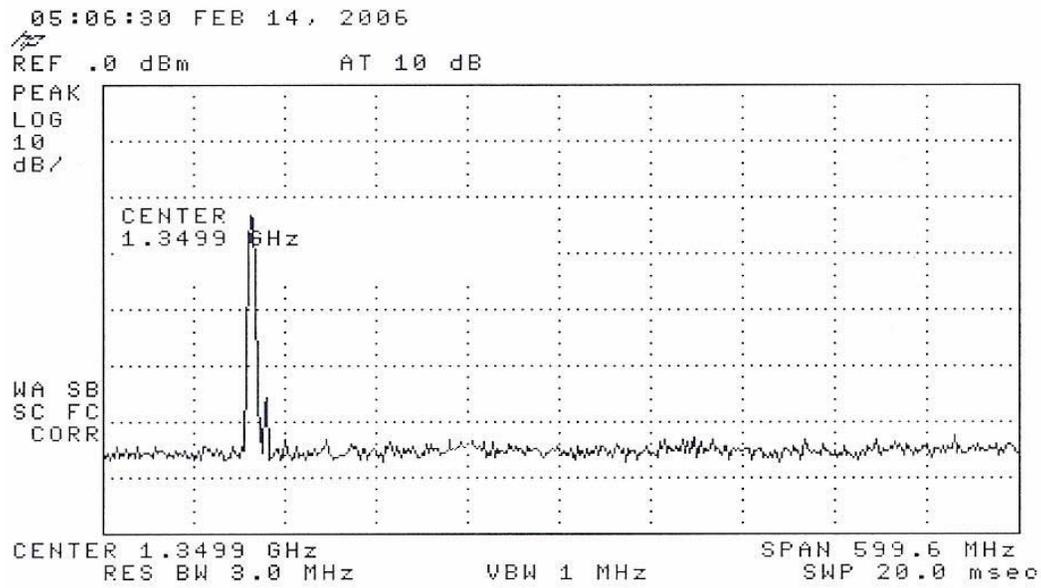
This set of experiments shows that there is a difference in the bioactivity between GSM 900 MHz and DCS 1800 MHz and this difference is mainly due to the higher intensity of GSM 900 under the same exposure conditions, (differences between groups 900 and 900A) and not due to the different RF carrier frequencies, (differences between 900A and 1800 groups).

Intensity differences between the two types of cellular mobile telephony radiation depend also on the ability of communication between the antennas of the mobile phone and the corresponding base station. Even if GSM 900 usually has a higher intensity than DCS 1800, this situation can be reversed in certain places if GSM 900 has a much better signal perception between its antennas than DCS 1800, (Tisal 1998). Our results count for equal signal perception conditions between the two types of radiation.

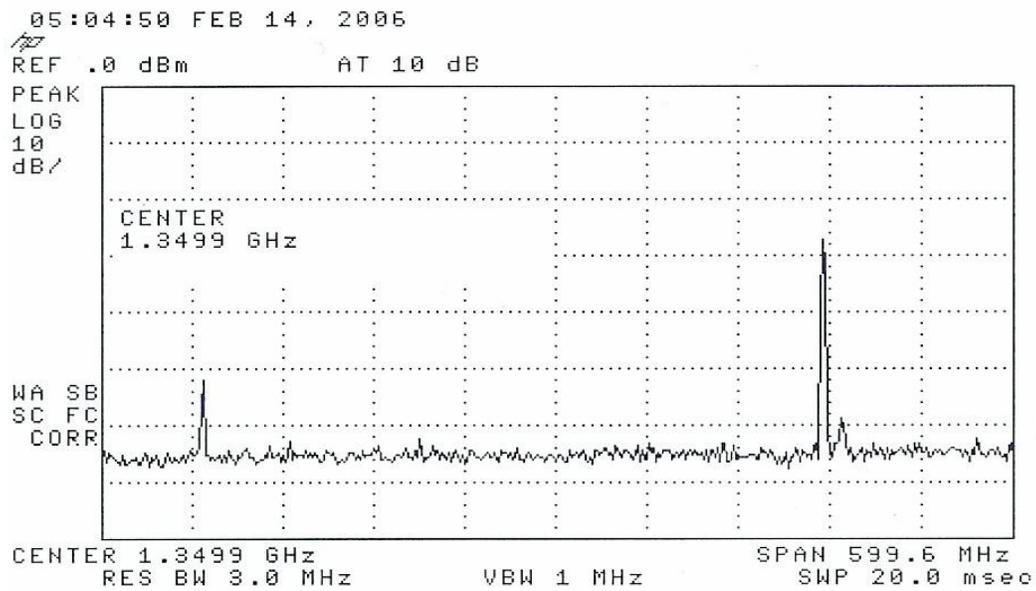


a. Background spectrum.

Figure 3. Continued on next page.



b. Spectrum of GSM 900 MHz.



c. Spectrum of DCS 1800 MHz.

Figure 3. Background, GSM 900 MHz and DCS 1800 MHz spectra.

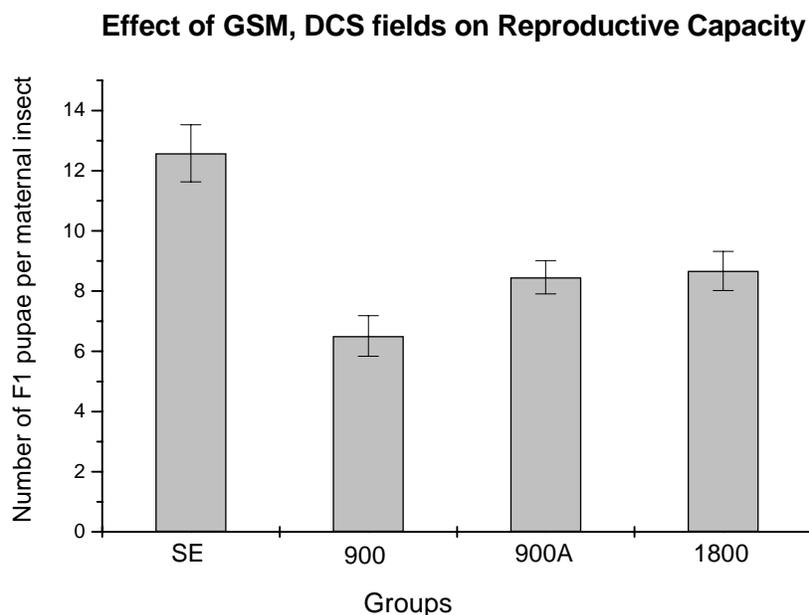


Figure 4. Reproductive Capacity (mean number of F1 pupae per maternal fly) of exposed (900, 900A, 1800) and sham exposed (SE) groups.

Table 4. Effect of Modulated GSM and DCS fields on the Reproductive Capacity of *Drosophila melanogaster*

Experiment No	Groups	Mean Number of F ₁ Pupae per Maternal Fly	Deviation from Control
1	900	7.7	-42.54%
	900A	8.9	-33.58%
	1800	9.2	-31.34%
	SE (Control)	13.4	
2	900	5.8	-51.26%
	900A	8.1	-31.93%
	1800	7.9	-33.61%
	SE (Control)	11.9	
3	900	6.8	-46.03%
	900A	7.9	-37.30%
	1800	8.7	-30.95%
	SE (Control)	12.6	
4	900	7.4	-47.52%
	900A	9.7	-31.21%
	1800	9.9	-29.79%
	SE (Control)	14.1	
5	900	6.2	-52.31%
	900A	8.5	-34.62%
	1800	8.2	-36.92%
	SE (Control)	13	

Table 4. Continued

Experiment No	Groups	Mean Number of F ₁ Pupae per Maternal Fly	Deviation from Control
6	900	6.1	-43.52%
	900A	8.2	-24.07%
	1800	7.8	-27.78%
	SE (Control)	10.8	
7	900	6.7	-47.66%
	900A	8.3	-35.16%
	1800	9	-29.69%
	SE (Control)	12.8	
8	900	6	-48.72%
	900A	7.9	-32.48%
	1800	8.4	-28.21%
	SE (Control)	11.7	
9	900	6.7	-49.24%
	900A	8.8	-33.33%
	1800	9.1	-31.06%
	SE (Control)	13.2	
10	900	5.7	-53.66%
	900A	8.3	-32.52%
	1800	8.5	-30.89%
	SE (Control)	12.3	
Average \pm SD	900	6.51 \pm 0.67	-48.25%
	900A	8.46 \pm 0.55	-32.75%
	1800	8.67 \pm 0.65	-31.08%
	SE (Control)	12.58 \pm 0.95	

5. Radiation Bioactivity According to its Intensity (or According to the Distance from the Antenna)

The aim of this set of experiments was to investigate the dependence of GSM 900 MHz and DCS 1800 MHz bioactivity on their intensity, at different intensity levels that people are exposed to, from mobile phones and base station antennas. The radiation from base station antennas is almost identical to that of corresponding mobile phones but it is about 100 times stronger. Thus distances from mobile phones antennas correspond to about 100 times longer distances from base station antennas of the same type of radiation.

It is difficult to set up experiments regarding exposures from base station antennas since there is no way to have a sham exposed group of experimental animals under identical environmental conditions but without being exposed to the radiation at the same time. Thus we thought that the only way to simulate the reality of the exposure by a base station antenna is to expose the animals at different distances from a mobile phone within the lab.

Biological effects of mobile telephony signals at different intensities- distances from the antenna of a mobile phone handset, resembling effects from base station signals within residential areas, were not performed until now.

In each single experiment of this set, we separated the collected insects into thirteen groups: The first group (named "0") was exposed to GSM 900 MHz or to DCS 1800 MHz

field with the mobile phone antenna in contact with the glass vial containing the flies. The second (named “1”), was exposed to GSM 900 MHz or to DCS 1800 MHz field, at 1cm distance from the mobile phone antenna. The third group (named “10”) was exposed to GSM 900 MHz or to DCS 1800 MHz field at 10 cm distance from the mobile phone antenna. The fourth group (named “20”) was exposed to GSM 900 MHz or to DCS 1800 MHz field at 20 cm distance from the mobile phone antenna, etc, the twelfth group (named “100”) was exposed to GSM 900 MHz or to DCS 1800 MHz field at 100 cm distance from the mobile phone antenna. Finally, the thirteenth group (named “SE”) was the sham exposed. Each group consisted of ten male and ten female insects as previously.

Radiation and field measurements in contact and at different distances from the mobile phone antenna, for six min of modulated emission, for GSM 900 MHz and DCS 1800 MHz in the RF and ELF ranges excluding the background electric and magnetic fields of 50 Hz, are given in Table 5. All the values shown in Table 5, are averaged over ten separate measurements of each kind \pm standard deviation (S.D.).

The measurements reveal that although ELF electric and magnetic fields fall at almost zero levels for distances longer than 50 cm from both GSM 900 and DCS 1800 mobile phone antennas, the RF components of the signals are still evident for distances up to 100 cm, (Table 5).

The Average mean values of reproductive capacity (number of F₁ pupae) from six identical experiments with each kind of radiation are shown in Table 6 and represented in Figures 5, 6. The statistical analysis (single factor Anova test) shows that the probability that the reproductive capacity differs between groups, owing to random variations, is negligible, $P < 10^{-8}$. Once again there was no temperature increases within the vials during the exposures.

The results show that the effect of mobile telephony radiation is maximum at zero distance (intensities higher than 200 $\mu\text{W}/\text{cm}^2$) and then becomes maximum at a distance of 20-30 cm from the antenna, depending on the intensity of radiation (GSM or DCS). This distance corresponds to an intensity around 10 $\mu\text{W}/\text{cm}^2$ for both types of radiation in regards to the RF components.

Table 5. Radiation and Field Intensities in the Microwave and ELF regions

Distance from Antenna (cm)	GSM Radiation Intensity at 900 MHz, (mW/cm^2)	GSM Electric Field Intensity at 217 Hz, (V/m)	GSM Magnetic Field Intensity at 217 Hz, (mG)	DCS Radiation Intensity at 1800 MHz, (mW/cm^2)	DCS Electric Field Intensity at 217 Hz, (V/m)	GSM Magnetic Field Intensity at 217 Hz, (mG)
0	0.380 \pm 0.058	19 \pm 2.5	0.9 \pm 0.15	0.250 \pm 0.048	13 \pm 2.1	0.6 \pm 0.08
1	0.260 \pm 0.047	12 \pm 1.7	0.7 \pm 0.13	0.068 \pm 0.015	6 \pm 0.8	0.4 \pm 0.07
10	0.062 \pm 0.020	7 \pm 0.8	0.3 \pm 0.05	0.029 \pm 0.005	2.9 \pm 0.48	0.2 \pm 0.05
20	0.032 \pm 0.008	2.8 \pm 0.4	0.2 \pm 0.04	0.012 \pm 0.002	0.7 \pm 0.12	0.1 \pm 0.02
30	0.010 \pm 0.002	0.6 \pm 0.09	0.1 \pm 0.02	0.007 \pm 0.001	0.3 \pm 0.06	0.06 \pm 0.01
40	0.006 \pm 0.001	0.2 \pm 0.03	0.05 \pm 0.01	0.004 \pm 0.0007	0.1 \pm 0.04	0
50	0.003 \pm 0.0006	0.1 \pm 0.02	0	0.002 \pm 0.0003	0	0
60	0.002 \pm 0.0003	0	0	0.0016 \pm 0.0002	0	0
70	0.0017 \pm 0.0002	0	0	0.0014 \pm 0.0002	0	0
80	0.0012 \pm 0.0002	0	0	0.0008 \pm 0.0002	0	0
90	0.0010 \pm 0.0001	0	0	0.0005 \pm 0.0001	0	0
100	0.0004 \pm 0.0001	0	0	0.0002 \pm 0.0001	0	0

Table 6. Effect of Modulated GSM and DCS radiation-fields on the Reproductive Capacity at different Distances-Intensities from the antenna

Groups -Distance from mobile phone antenna, (cm)	Average Mean Number of F ₁ Pupae per Maternal Fly, for GSM 900 MHz	Deviation from Sham Exposed Group	Average Mean Number of F ₁ Pupae per Maternal Fly, for DCS 1800 MHz	Deviation from Sham Exposed Group
0	7.45 ± 0.72	-46.01 %	9.26 ± 0.68	-34.00 %
1	9.38 ± 0.61	-32.03 %	11.36 ± 0.54	-19.03 %
10	11.29 ± 0.80	-18.19 %	11.93 ± 0.71	-14.97 %
20	11.52 ± 0.79	-16.52 %	9.19 ± 0.62	-34.50 %
30	7.33 ± 0.58	-46.88 %	13.03 ± 0.83	-7.13 %
40	12.88 ± 0.98	-6.67 %	13.76 ± 0.85	-1.92 %
50	13.48 ± 0.81	-2.32 %	13.85 ± 0.74	-1.28 %
60	13.61 ± 0.84	-1.38 %	14.00 ± 0.91	-0.21 %
70	13.70 ± 0.91	-0.72 %	14.21 ± 0.89	+1.28 %
80	13.97 ± 0.77	+1.23 %	14.07 ± 0.79	+0.29 %
90	13.74 ± 0.96	-0.43 %	14.02 ± 1.03	-0.07 %
100	14.02 ± 1.01	+1.59 %	14.31 ± 1.08	+2.00 %

Intensity Effect of GSM 900 MHz Radiation

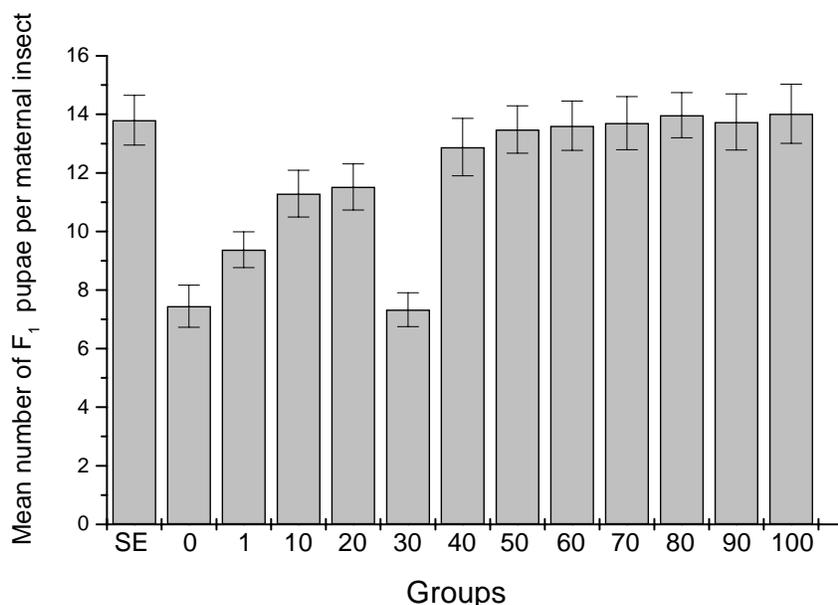


Figure 5. Reproductive Capacity in relation to the Distance from a GSM 900 MHz mobile phone antenna. The decrease in reproductive capacity is maximum at zero distance and at 30 cm distance from the antenna, corresponding to RF intensities 380 μ W/cm² and 10 μ W/cm² (Table 5).

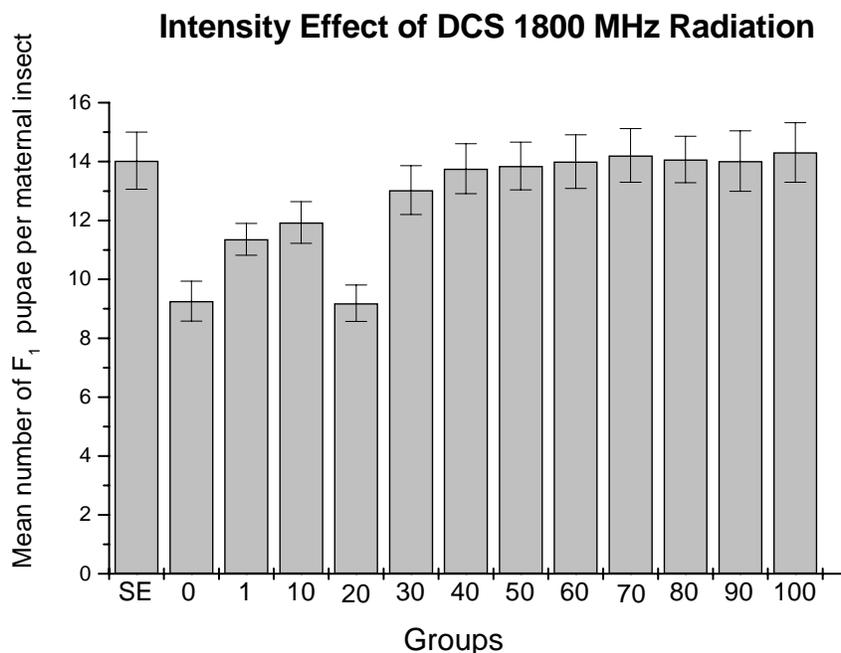


Figure 6. Reproductive Capacity in relation to the Distance from a DCS 1800 MHz mobile phone antenna. The decrease in reproductive capacity is maximum at zero distance and at 20 cm distance from the antenna, corresponding to RF intensities $250 \mu\text{W}/\text{cm}^2$ and $12 \mu\text{W}/\text{cm}^2$ (Table 5).

The effect on the reproductive capacity diminishes considerably for distances longer than 50 cm from the mobile phone antenna and disappears for distances longer than 80-90 cm, corresponding to radiation intensities smaller than $1 \mu\text{W}/\text{cm}^2$. For distances longer than 50 cm where the ELF components fall within the background, the decrease in reproductive capacity is within the standard deviation. This might suggest that the ELF components of digital mobile telephony signals, play a key role in their bio-activity, alone or in conjunction with the RF carrier wave.

We have recorded the existence of an “intensity window” around $10 \mu\text{W}/\text{cm}^2$ (in regards to the RF intensity) where the bio-effect becomes even more intense than at intensities higher than $200 \mu\text{W}/\text{cm}^2$. This intensity window appears at a distance of 20-30 cm from a mobile phone antenna, which corresponds to a distance of about 20-30 meters from a base station antenna. Since mobile telephony base station antennas are usually located within residential areas, at distances 20-30 m from such antennas there are often houses and work places where people are exposed up to 24 hours per day.

Although intensity windows on the bio-effects of RF radiations have been recorded since many years, (Bawin et al 1975; 1978; Blackman et al, 1980), there is still no widely accepted explanation for their existence.

6. The Decrease in Reproductive Capacity is due to Cell Death in the Gonads

In each experiment of this final sixth set, we separated the collected insects into five groups. The first four groups were the same just as in the No 4 experiments: The first group (named

“900”) was exposed to GSM 900 MHz field with the mobile phone antenna in contact with the glass vial containing the flies. The second (named “900A”), was exposed to GSM 900 MHz at 1cm distance from the mobile phone antenna. The third group (named “1800”) was exposed to DCS 1800 MHz field with the mobile phone antenna in contact with the glass vial. The fourth group (named “SE”) was sham-exposed. Finally there was an additional fifth group (named “C”) which was the control. While sham-exposed animals were treated exactly as the exposed ones except that the mobile phone was turned off during the “exposures”, control animals were never exposed in any way or even taken out of the culture room. Each group consisted as always of ten male and ten female insects.

In this set of experiments, there was an additional 6 min exposure in the morning of the sixth day, and one hour later female insects from each group were dissected and prepared for TUNEL assay. This additional exposure time was the only difference in the exposure procedure from the previous sets of experiments. Since we were studying the effect on early and mid oogenesis during which the egg chambers develop from one stage to the next within few hours, (King, 1970), an additional exposure, one hour before dissection and fixation of the ovarioles, was proven to be important in recording immediate effects on DNA fragmentation.

The most anterior region of the ovariole is called the germarium. The most sensitive developmental stages during oogenesis for stress-induced apoptosis, are region 2 within the germarium referred to as “germarium checkpoint” and stages 7-8 just before the onset of vitellogenesis, referred to as “mid-oogenesis checkpoint”, (Drummond-Barbosa and Spradling, 2001; McCall 2004). The nurse cells (NC) and follicle cells (FC) of both checkpoints, were found to be very sensitive to stress factors like poor nutrition, (Drummond-Barbosa and Spradling, 2001; Smith et al., 2002), or exposure to cytotoxic chemicals like etoposide or staurosporine, (Nezis et al., 2000). Apart from these two check points, egg chambers were not observed before to degenerate during other provitellogenic or vitellogenic stages, (germarium to stage 10), (Drummond-Barbosa and Spradling, 2001; McCall 2004).

To determine the ability of GSM and DCS radiation to act as possible stress factors able to induce cell death during early and mid oogenesis, we used TUNEL assay, as described above. The samples from different experimental groups were blindly observed under the fluorescence microscope (i.e. the observer did not know the origin of the sample) and the percentage of egg chambers with TUNEL positive signal was scored in each sample. Statistical analysis was made by single factor Analysis of Variance test.

In Table 7 the summarised data from 8 separate experiments are listed. The data reveal that both GSM 900 and DCS 1800 mobile telephony radiations strongly induce cell death, (DNA fragmentation) in ovarian egg chambers of the exposed groups, (63.01% in 900, 45.08% in 900A and 39.43% in 1800), while in the SE and C groups the corresponding percentage of cell death was only 7.78% and 7.75% respectively.

Ovarian cell death between the control group and the sham exposed group did not differ significantly, (differences were within standard deviation) and this is why the data from the C group are omitted in Table 7.

Electromagnetic stress from mobile telephony radiations was found in our experiments to be much more bioactive than previously known stress factors like poor nutrition or cytotoxic chemicals, inducing cell death to a higher degree not only to the above check points but to all developmental stages of early and mid oogenesis and moreover to all types of egg chamber cells, i.e. nurse cells, follicle cells and the oocyte (OC), (Panagopoulos et al, 2007a).

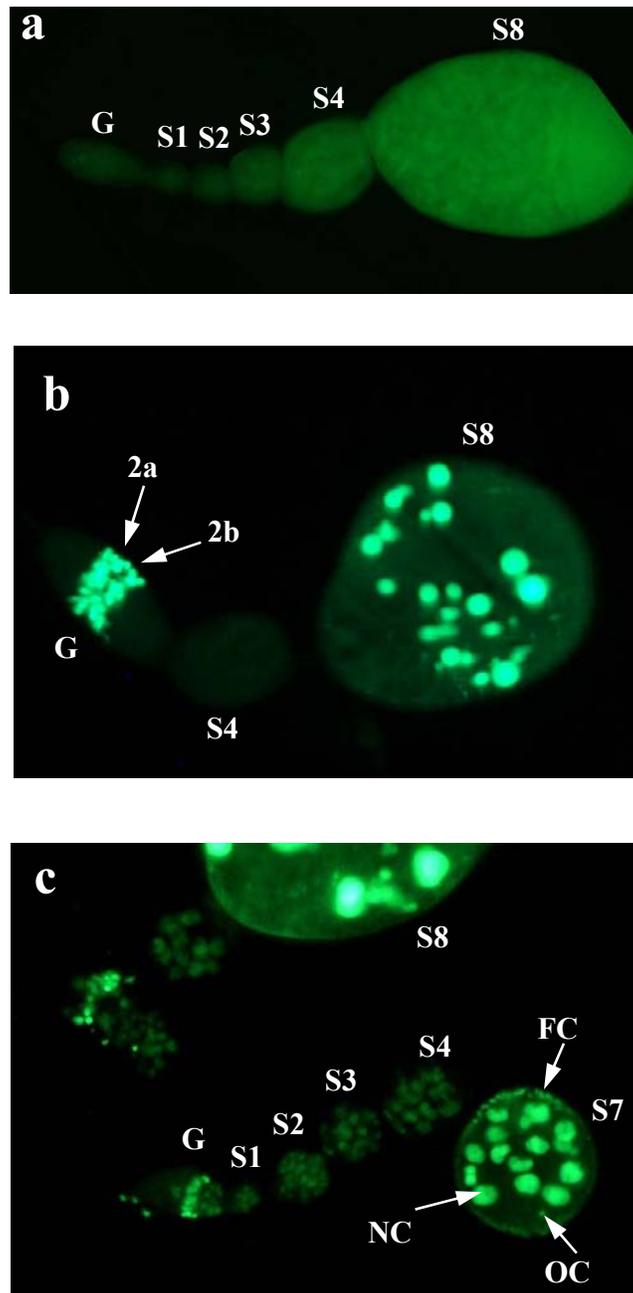


Figure 7. a) Ovariole of a sham exposed female insect with TUNEL negative egg chambers at all the developmental stages from germarium (G) to stage 8. b) Ovariole of exposed female insect with TUNEL positive signal at both check-points, germarium and stage 8 and TUNEL negative signal at the intermediate stages. c) Ovarioles of exposed female insects with TUNEL positive signals at all the developmental stages and in all types of egg chamber cells, nurse cells (NC), follicle cells (FC) and the oocyte (OC).

Table 7. Effect of GSM, DCS fields on Ovarian Cell Death

Groups	Dev. Stages	Ratio of TUNEL Positive to Total Number of Egg-chambers of each dev. stage	Sum Ratio of TUNEL Positive to Total Number of Egg-chambers of all stages	Percentage of TUNEL Positive Egg chambers	Deviation from Sham Exposed Groups
SE	Germarium	37/186	154/1980	7.78%	0%
	1-6	32/1148			
	7-8	78/364			
	9-10	7/282			
900	Germarium	165/189	1315/2087	63.01%	+55.23%
	1-6	675/1252			
	7-8	310/384			
	9-10	165/262			
900A	Germarium	116/184	930/2063	45.08%	+37.30%
	1-6	484/1248			
	7-8	213/374			
	9-10	117/257			
1800	Germarium	101/169	776/1968	39.43%	+31.65%
	1-6	388/1202			
	7-8	196/358			
	9-10	91/239			

Figure 7a, shows an ovariole from a sham exposed female insect, containing egg chambers from germarium to stage 8, all TUNEL negative. This was the typical picture in the vast majority of ovarioles and separate egg chambers from female insects of the sham exposed and control groups. In the SE groups, only 154 egg chambers (including germaria) out of a total of 1980 in 8 replicate experiments (7.78%), were TUNEL positive (Table 7), a result that is in full agreement with the rate of spontaneously degenerated egg chambers normally observed during *Drosophila* oogenesis, (Nezis et al., 2000; Baum et al., 2005).

Figure 7b shows an ovariole of exposed female insect (group 900A), with a TUNEL positive signal in the nurse cells at both checkpoints, germarium and stage 8, while egg chambers of intermediate stages are TUNEL negative. Corresponding pictures from 900 and 1800 (data not shown) had identical characteristics. The two checkpoints in all groups (exposed and SE/C) had the highest percentages of cell death compared to the other developmental stages 1-6 and 9-10, (Table 7). While in the SE groups the sum ratio of TUNEL positive to total number of egg chambers was slightly higher in stages 7-8 (78/364) than in the germarium (37/186), in all three exposed groups this ratio was higher in the germarium than in stages 7-8, (Table 7).

Figure 7c, shows ovarioles of exposed female insects (group 900A), with a TUNEL positive signal at all developmental stages from germarium to 7-8 and in all the cell types of the egg chamber, (nurse cells, follicle cells and the oocyte).

Although in most pictures the TUNEL positive signal was most evident in the nurse cells, in the majority of the egg chambers in all the exposed groups, a TUNEL positive signal was detected in all three kinds of egg chamber cells, (figures 1c).

Ovarian Cell Death induced by GSM and DCS Radiations

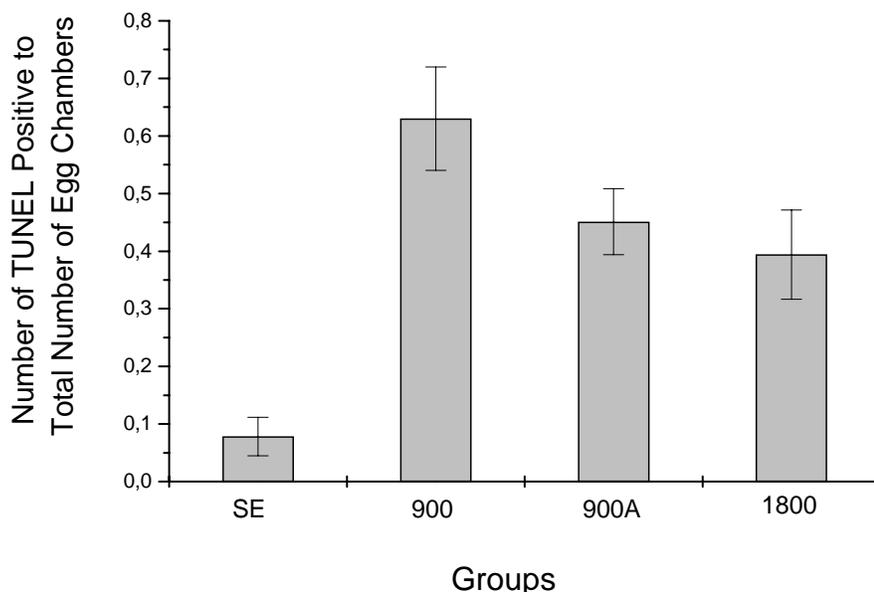


Figure 8. Mean ratio of Ovarian Cell Death (Number of TUNEL Positive to Total Number of Egg Chambers), in each experimental group \pm SD, (0.078 ± 0.0335 in SE, 0.630 ± 0.0898 in 900, 0.451 ± 0.0574 in 900A and 0.394 ± 0.0777 in 1800).

In the SE groups the ratio of TUNEL positive egg chambers of stages 9-10 was very small (7/282). In contrast, the corresponding ratio in all three exposed groups was significantly higher, (165/262 in 900, 117/257 in 900A and 91/239 in 1800).

The summarised data of Table 7 are represented in Fig.8.

The statistical analysis, (single factor Analysis of Variance test), showed that the probability that groups differ between them because of random variations, is negligible, $P < 10^{-13}$.

Our experiments and the statistical analysis show that genomic DNA fragmentation of the egg chambers cells is induced by the mobile telephony radiation. Both types of radiation, GSM 900MHz and DCS 1800MHz induce cell death in a large number (up to 55% in relation to control), of ovarian egg chambers in the exposed insects with only 6 min exposure per day for a limited period of 6 days.

DNA fragmentation is induced in all cases predominantly at the two developmental stages named checkpoints, germarium and stages 7-8. Since the above check points were already known to be the most sensitive stages in response to other stress factors, (Chao and Nagoshi 1999; De Lorenzo et al., 1999; Nezis et al., 2000; Drummond-Barbosa and Spradling 2001; McCall 2004), such an observation could be expected. Our results show that these two checkpoints are the most sensitive stages also in response to electromagnetic stress. However the germarium checkpoint was found to be even more sensitive than stages 7-8 in response to this particular stress. Thereby the two check points are not equally responsive to distinct types of stress and may therefore also respond differentially to other types of stress stimuli. A possible explanation for the more sensitive germarium stage is that it may be more effective

in evolutionary terms for the animal to block development of any defective egg chamber at the beginning rather than at later stages, in order to prevent the waste of precious nutrients.

In the sham exposed/control groups, induced DNA fragmentation was observed almost exclusively at the two developmental stages named check-points (37/186 in the germarium and 78/364 in stage 7-8) and only in few cases at the other provitellogenic and vitellogenic stages, 1-6 (32/1148) and 9-10 (7/282), correspondingly. In contrast, ovarian egg chambers of animals from all three exposed groups, were found to be TUNEL positive to a high degree at all developmental stages from germarium to stage 10, (Table 7).

In all cases (both in the sham exposed/control and also in the exposed groups), the TUNEL positive signal was more intense at the two check points, germarium and stages 7-8, than at the other developmental stages.

There was no detectable temperature increase within the vials during the exposures, therefore the effects are considered as non-thermal.

In this set of experiments, cell death was detected for the first time during all the developmental stages of early and mid oogenesis in *Drosophila*, from germarium to stage 10 and in all types of egg chamber cells, (nurse cells, follicle cells, oocyte). A possible explanation for these effects is that the electromagnetic stress induced in the ovarian cells by the GSM and DCS fields, is a new and probably more intense type of external stress, against which ovarian cells do not have adequate defence mechanisms like they do in the case of poor nutrition or chemical stress.

It is important to emphasize that the recorded effect in the oocyte which undergoes meiosis during the last stages of oogenesis, may result in heritable mutations upon DNA damage induction and repair, if not in cell death.

The results of this set of experiments reveal that the large decrease of reproductive capacity found in the previous sets of experiments is due to elimination of large numbers of egg chambers during early and mid oogenesis, either via stress induced apoptosis or necrosis of their constituent cells, caused by the mobile telephony radiation.

Our present results are in agreement with results of other experimenters reporting DNA damage in other cell types, assessed by different methods than ours, after *in vivo* or *in vitro* exposure to GSM radiation, (Diem et al., 2005; Markova et al., 2005; Salford et al., 2003; Lai and Singh 1995; 1996).

We do not know if the ovarian cell death found in our experiments to be induced by mobile telephony radiation is due to apoptosis, i.e. caused by the organism in response to the electromagnetic stress, or the result of necrosis caused directly by the electromagnetic radiation. This important issue remains to be uncovered.

A Plausible Mechanism for Mobile Telephony Radiation Bioeffects

As we have previously reported, (Panagopoulos et al. 2000b; 2002; Panagopoulos and Margaritis 2003b), any external oscillating electromagnetic field can induce a forced-vibration on the free ions that exist in large concentrations inside and outside all living cells in biological tissue playing a key role in all cellular functions initiating or accompanying all cellular biochemical processes.

The forced-vibrational movement of the free ions is described by the equation,

$$m_i \frac{d^2x}{dt^2} + \lambda \frac{dx}{dt} + m_i \omega_o^2 x = E_o z q_e \sin \omega t \quad [1]$$

in the case of an external harmonically oscillating electric field: $E = E_o \sin \omega t$ with circular frequency: $\omega = 2\pi\nu$, (ν , the frequency), where: z is the ion's valence, $q_e = 1.6 \times 10^{-19}$ Cb, the electron's charge, $F_2 = -m_i \omega_o^2 x$, a restoration force proportional to the displacement distance x of the free ion, m_i the ion's mass and $\omega_o = 2\pi\nu_o$, with ν_o the ion's oscillation self-frequency if the ion were left free after its displacement x . In our case, this restoration force is found to be very small compared to the other forces and thus does not play any important role. $F_3 = -\lambda u$ is the damping force, where $u = \frac{dx}{dt}$, is the ion's velocity and λ , is the attenuation coefficient for the ion's movement, which for the cytoplasm or the extracellular medium is calculated to be $\lambda \cong 10^{-12}$ Kg/sec, while for ions moving inside channel proteins, is calculated to have a value: $\lambda \cong 6.4 \times 10^{-12}$ Kg/sec, (in the case of Na^+ ions, moving through open Na^+ channels), (Panagopoulos et al 2000b).

We have shown that the general solution of equation [1], is:

$$x = \frac{E_o z q_e}{\lambda \omega} \cos \omega t - \frac{E_o z q_e}{\lambda \omega} \quad [2]$$

Since the second term of [2] is constant, the vibrational movement is described by the equation:

$$x = \frac{E_o z q_e}{\lambda \omega} \cos \omega t \quad [3]$$

Eq. [3] shows that the forced - vibration is in phase with the external force. The amplitude of the free ions forced vibration is,

$$A = \frac{E_o z q_e}{\lambda \omega} \quad [4]$$

Thus, the amplitude is proportional to the intensity and inversely proportional to the frequency of the external oscillating field.

Once this amplitude exceeds some critical value the coherent forces that the ions exert on the voltage sensors of voltage-gated membrane channels can trigger the irregular opening or closing of these channels, thus disrupting cell's electrochemical balance and function.

We have shown that in the most bioactive case of pulsed fields and for double valence cations (i.e. Ca^{+2}) interacting with the channel sensor, the condition for irregular gating of the channel becomes:

$$E_o \geq \nu \times 0.625 \times 10^{-4} \quad [5]$$

(ν in Hz, E_o in V/m). Whenever [5] is satisfied, the external field E can irregularly gate the ion channel.

Relation [5] declares that external ELF electric fields with intensities less than tenths of a mV/m should theoretically be able to disrupt cell function by irregular gating of ion channels (!)

According to this mechanism, lower frequency fields are the most bioactive ones and additionally pulsed fields are shown to be more bioactive than continuous, (uninterrupted), ones, (Panagopoulos et al., 2002).

Thereby, the ELF components of the mobile telephony signals are certainly within the criteria of this theory and thus able to produce the reported effects on living organisms.

Somebody may wonder, how could be possible that irregular gating of ionic channels on a cell membrane could lead to cell death.

Let us consider the irregular gating of ion channels on a cell's plasma membrane. If the electrochemical balance is destroyed by irregular increase of intracellular ion concentration, then water molecules may enter the cell driven by osmotic forces, proportional to the concentration increase. Such an effect could be able to cause the cell to swell out and the plasma membrane to get ruptured, resulting to cell necrosis.

It is known that perturbations of intracellular Ca^{+2} concentrations are responsible for apoptotic triggering, (Zhou et al., 1998; Sheikh and Huang, 2004; Santini et al. 2005). Therefore, another scenario of cell death, caused by irregular gating of ion channels, could be that due to altered intracellular Ca^{+2} concentrations, a false signal may be given to initiate apoptosis.

A common event leading to both apoptosis and necrosis is mitochondrial membrane permeabilization, (Armstrong 2006). This can also be done by direct action of an external EMF on mitochondrial membrane Ca^{+2} channels. Apoptosis is connected with increased mitochondrial concentration of Ca^{+2} ions, released from the endoplasmic reticulum, (Santini et al., 2005). A false uptake of Ca^{+2} ions by mitochondria can be due to irregular opening of mitochondrial Ca^{+2} channels, or due to increased cytosolic Ca^{+2} concentration, caused by irregular release either through the membrane of endoplasmic reticulum or through the plasma membrane. In all cases this could be done by irregular gating of electrosensitive Ca^{+2} channels which exist in all cell membranes.

We have just described few of the many hypothetical but very possible biochemical scenarios which could very explain by means of the above described biophysical theory, the effects of DNA damage recorded in our experiments as well as in other labs experiments, (Diem et al., 2005; Markova et al., 2005; Salford et al., 2003; Lai and Singh 1995; 1996).

Conclusions

As shown by increasing number of biological, clinical and epidemiological studies, the radiations emitted by mobile telephony, at levels that people are daily exposed, are highly bioactive producing a variety of effects on living organisms.

Our studies regarding the effects of mobile telephony radiations on a biological model, the reproductive capacity of the insect *Drosophila melanogaster*, have investigated different

physical parameters of these radiations, like intensity, carrier frequency, pulse repetition frequency, distance from the antenna, e.t.c.

Our experiments have shown a large decrease in reproductive capacity caused by the GSM and DCS fields-radiation. The recorded effect is due to extensive DNA fragmentation on reproductive cells of the experimental animal, induced by these fields-radiation.

Thus, digital mobile telephony radiations nowadays exert an intense biological action able to kill cells, damage DNA, or decrease dramatically the reproductive capacity of living organisms. Diminishes of bird and insect populations can be explained according to reproduction decreases. Phenomena like headaches, fatigue, sleep disturbances, memory loss e.t.c. reported as “microwave syndrome” can possibly be explained by cell death on a number of brain cells during daily exposures from mobile telephony antennas.

Our experiments show that radiation intensities higher than $1 \mu\text{W}/\text{cm}^2$ are able to decrease reproduction of living organisms by killing reproductive cells. Our opinion is that the international exposure limits for these radiations should be set not higher than $1 \mu\text{W}/\text{cm}^2$. Since short term exposures for few minutes per day are able to produce so intense effects on living organisms, the criteria should not be set according to average values but according to maximum values during the exposure periods.

Our experiments reveal that exposure at a distance of 20-30 cm from a mobile phone can be even more bioactive than exposure in contact with the antenna, due to the existence of an “intensity window” around $10 \mu\text{W}/\text{cm}^2$. This intensity, in the case of a usual base station antenna corresponds to a distance of about 20-30 m from the antenna.

Although both types of radiation examined are found to be highly bioactive, GSM 900 MHz seems to be even more bioactive than DCS 1800 MHz, mainly due to higher intensity, but also even when it is emitted at almost the same intensity. Since differences in bioactivity between the two types of radiation under the same intensity are within standard deviation, it seems that RF carrier frequency plays a minimal role in the bioactivity of this radiation, in contrast to the ELF pulse repetition frequencies and the radiation and field intensities that seem to be of great importance in regards to bioactivity.

The ELF components of the mobile telephony signals, seem to play a key role on their bio-effects, since the recorded effects are considerably diminished at distances that these components fall within the background of stray 50 Hz electric and magnetic fields. This supports that lower frequency fields are more bioactive than higher frequency ones with the same rest characteristics, as it is predicted by our theory, (Panagopoulos et al 2000b; 2002), and supported by other experimental evidence, (Lin Liu and Adey 1982; Penafiel et al 1997).

A plausible explanation of the effects of mobile telephony radiations on living organisms is given by the biophysical mechanism that we have proposed, (Panagopoulos et al. 2000b; 2002; Panagopoulos and Margaritis 2003b). According to this mechanism, altered intracellular ionic concentrations due to irregular gating of ion channels on the cell membranes by an external electromagnetic field can initiate cell death through apoptosis or necrosis.

Similar effects on humans with those recorded in our experiments on insects, are considered to be possible because first, insects are found to be more resistant to radiations than mammals, (Koval and Kazmar 1988, Koval et al 1979, 1977, Abrahamson et al 1973) and second, our results are in agreement with reported effects on mammals, (Lai and Singh 1995; 1996; Aitken et al., 2005; Salford et al., 2003).

Scientific evidence implies the need of reconsideration of the current exposure criteria to account for non-thermal effects which constitute the large majority of the recorded biological and health effects. Since Mobile Telephony has become part of our daily life, a better design of base station antenna networks towards the least exposure of residential areas and a very cautious use of mobile phones, is necessary.

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